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 * * * * * * * * * * * * * STN  Columbus * * * * * * * * * * * * * * *
FILE 'HOME' ENTERED AT 11:54:50 ON 25 JUN 2003
=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
=> e rikihisa y/au
E1        26    RIKIHISA W/AU
E2         5    RIKIHISA WATARU/AU
E3       910 --> RIKIHISA Y/AU
E4        15    RIKIHISA Y */AU
E5         2    RIKIHISA Y O/AU
E6         1    RIKIHISA Y T/AU
E7         3    RIKIHISA YASUHIRO/AU
E8         1    RIKIHISA YASUKI/AU
E9       189    RIKIHISA YASUKO/AU
E10        1    RIKIHISAB Y/AU
E11        1    RIKIHISAL Y/AU
E12        4    RIKIHITO/AU

=> s e3-e9 and ehrlich?
L1       744 ("RIKIHISA Y"/AU OR "RIKIHISA Y *"/AU OR "RIKIHISA Y O"/AU OR
          "RIKIHISA Y T"/AU OR "RIKIHISA YASUHIRO"/AU OR "RIKIHISA YASUKI"
          /AU OR "RIKIHISA YASUKO"/AU) AND EHRLICH?

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2       231 DUP REM L1 (513 DUPLICATES REMOVED)

=> s l2 and P30?
L3        14 L2 AND P30?

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 14 ANSWERS - CONTINUE? Y/(N):y


L3  ANSWER 1 OF 14  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN  2003:217479  BIOSIS
DN  PREV200300217479
TI  Outer membrane protein of  ***Ehrlichia***  canis and  ***Ehrlichia***
    chaffeensis.
AU  ***Rikihisa, Yasuko***  ; Ohashi, Norio
    ASSIGNEE: The Ohio State University Research Foundation
PI  US 6544517 April 08, 2003
SO  Official Gazette of the United States Patent and Trademark Office Patents,
    (Apr. 8 2003) Vol. 1269, No. 2, pp. No Pagination.
    http://www.uspto.gov/web/menu/patdata.html. e-file.
    ISSN: 0098-1133.
```

DT Patent
LA English
AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. chaffeensis and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. canis and variants thereof referred to hereinafter as the " ***P30F*** , proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. chaffeensis encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The P3OF proteins of E. canis encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. chaffeensis OMP proteins and isolated polynucleotides that encode the E. canis ***P30F*** protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.

L3 ANSWER 2 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:501879 BIOSIS
DN PREV200200501879
TI Methods for detecting ***Ehrlichia*** canis and ***Ehrlichia*** chaffeensis in vertebrate and invertebrate hosts.
AU Stich, Roger William (1); ***Rikihisa, Yasuko***
CS (1) Columbus, OH USA
ASSIGNEE: The Ohio State University Research Foundation
PI US 6432649 August 13, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 13, 2002) Vol. 1261, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DT Patent
LA English
AB Tools and methods for detecting the presence of E. canis and E. chaffeensis in a sample obtained from an animal are provided. The methods employ a polymerase chain reaction and primer sets that are based on the ***p30*** gene of E. canis and the p28 gene of E. chaffeensis. The present invention also relates to the ***p30*** and the p28 primer sets. Each ***p30*** primer set comprises a first primer and the second primer, both of which are from 15 to 35 nucleotides in length. The first ***p30*** primer comprises a sequence which is complementary to

a consecutive sequence, within the following sequence: CCA AGTGTCTCAC ATTTTGGTAG CTTCTCAGCT AAAGAAGAAA GCAAATCAAC TGTTGGAGTTTTTGGATTAA AACATGATTG GGATGGAAGT CCAATACTTA AGAATAAACA CGCTGACTTTACTGTTCCAA AC. SEQ ID NO.1. The second ***p30*** primer comprises a sequence which is complementary to the inverse complement of a consecutive sequence contained within the following sequence: GTTACT CAATGGGTGG CCCAAGAATA GAATTCGAAA TATCTTATGA AGCATTCGAC GTAAAAAGTC CTAATATCAA TTATCAAAAT GACGCGCACA GGTACTGCGC TCTATCTCAT CACACATCGG CAGCCAT, SEQ ID NO.2. The first p28 comprises a sequence which is complementary to a consecutive sequenc, within the following sequence : A GTTTTCATAA CAAGTGCATT GATATCACTA ATATCTTCTC TACCTGGAGT ATCATTTTCC GACCCAACAG GTAGTGGTAT TAACGG, SEQ ID NO. 3. The second p28 primer comprises a sequence which is complementary to the inverse complement of a consecutive sequence within one of the following two sequences: CAT TTCTAGGTTT TGCAGGAGCT ATTGGCTACT CAATGGATGG TCCAAGAATA GAGCTTGAAG TATCTTATGA, SEQ ID NO. 4, or C AAGGAAAGTT AGGTTTAAGC TACTCTATAA GCCCAGA, SEQ ID NO. 5.

L3 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:504077 BIOSIS
DN PREV200100504077
TI Transcriptional analysis of ***p30*** major outer membrane multigene family of ***Ehrlichia*** canis in dogs, ticks, and cell culture at different temperatures.
AU Unver, Ahmet; Ohashi, Norio; Tajima, Tomoko; Stich, Roger W.; Grover, Debra; ***Rikihisa, Yasuko (1)***
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA
SO Infection and Immunity, (October, 2001) Vol. 69, No. 10, pp. 6172-6178. print.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB ***Ehrlichia*** canis, an obligatory intracellular bacterium of monocytes and macrophages, causes canine monocytic ***ehrlichiosis*** . E. canis immunodominant 30-kDa major outer membrane proteins are encoded by a polymorphic multigene family consisting of more than 20 paralogs. In the present study, we analyzed the mRNA expression of 14 paralogs in

experimentally infected dogs and Rhipicephalus sanguineus ticks by reverse transcription-PCR using gene-specific primers followed by Southern blotting. Eleven out of 14 paralogs in E. canis were transcribed in increasing numbers and transcription levels, while the mRNA expression of the 3 remaining paralogs was not detected in blood monocytes of infected dogs during the 56-day postinoculation period. Three different groups of R. sanguineus ticks (adult males and females and nymphs) were separately infected with E. canis by feeding on the infected dogs. In these pools of acquisition-fed ticks as well as in the transmission-fed adult ticks, the transcript from only one paralog was detected, suggesting the predominant transcription of that paralog or the suppression of the remaining paralogs in ticks. Expression of the same paralog was higher whereas expression of the remaining paralogs was lower in E. canis cultivated in dog monocyte cell line DH82 at 25degreeC than in E. canis cultivated at 37degreeC. Analysis of differential expression of ***p30*** multigenes in dogs, ticks, or monocyte cell cultures would help in understanding the role of these gene products in pathogenesis and E. canis transmission as well as in designing a rational vaccine candidate immunogenic against canine ***ehrlichiosis*** .

L3 ANSWER 4 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:379120 BIOSIS
DN PREV200000379120
TI Characterization and expression analysis of ***p30*** multigenes encoding major outer membrane proteins identified within two regions of ***Ehrlichia*** canis genome.
AU Ohashi, N. (1); Unver, A. (1); ***Rikihisa, Y. (1)***
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 242-243. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology Los Angeles, California, USA May 21-25, 2000 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English
SL English

L3 ANSWER 5 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:379119 BIOSIS
DN PREV200000379119
TI Expression analysis of multiple ***p30*** genes of ***Ehrlichia*** canis in dogs and ticks.
AU Unver, A. (1); Tajima, T. (1); Ohashi, N. (1); ***Rikihisa, Y. (1)*** ; Stich, R. W. (1)

CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 242. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology Los Angeles, California, USA May 21-25, 2000 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English
SL English

L3 ANSWER 6 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:324751 BIOSIS
DN PREV199900324751
TI Characterization of ***p30*** multigene family of ***Ehrlichia*** canis.
AU Ohashi, N. (1); ***Rikihisa, Y. (1)***
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 233.
Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English

L3 ANSWER 7 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:435123 BIOSIS
DN PREV199800435123
TI Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of ***Ehrlichia*** canis and application of the recombinant protein for serodiagnosis.
AU Ohashi, Norio; Unver, Ahmet; Zhi, Ning; ***Rikihisa, Yasuko (1)***
CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Journal of Clinical Microbiology, (Sept., 1998) Vol. 36, No. 9, pp. 2671-2680.
ISSN: 0095-1137.
DT Article
LA English
AB A 30-kDa major outer membrane protein of ***Ehrlichia*** canis, the agent of canine ***ehrlichiosis*** , is the major antigen recognized by both naturally and experimentally infected dog sera. The protein cross-reacts with a serum against a recombinant 28-kDa protein (rP28), one

of the outer membrane proteins of a gene (omp-1) family of ***Ehrlichia*** chaffeensis. Two DNA fragments of E. canis were amplified by PCR with two primer pairs based on the sequences of E. chaffeensis omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of E. canis. Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E. canis genome. Three copies of the entire gene (***p30*** , ***p30*** -1, and ***p30a***) were cloned and sequenced from the E. canis genomic DNA. The open reading frames of the two copies (***p30*** and ***p30*** -1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E. canis 30-kDa protein genes and the E. chaffeensis omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp., p44 from the agent of human granulocytic ***ehrlichiosis*** , msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. canis 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E. canis 30-kDa proteins are closely related but that the third 30-kDa protein is not. The ***p30*** gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified E. canis. Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 of E. canis. To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ***ehrlichiosis*** , the immunoreactions between rP30 and the whole purified E. canis antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IFA-positive and -negative plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ***ehrlichiosis*** . Cloning of multigenes encoding the 30-kDa major outer membrane proteins of E. canis will greatly facilitate understanding pathogenesis and immunologic study of canine ***ehrlichosis*** and provide a useful tool for phylogenetic analysis.

L3 ANSWER 8 OF 14 CABA COPYRIGHT 2003 CABI
AN 2003:76257 CABA
DN 20033045715
TI Transcriptional analysis of ***p30*** major outer membrane protein genes of ***Ehrlichia*** canis in naturally infected ticks and sequence analysis of ***p30*** -10 of E. canis from diverse geographic

regions
AU Felek, S.; Greene, R.; ***Rikihisa, Y.***
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH 43210-1093, USA.
SO Journal of Clinical Microbiology, (2003) Vol. 41, No. 2, pp. 886-888. 18 ref.
Publisher: American Society for Microbiology (ASM). Washington
ISSN: 0095-1137
CY United States
DT Journal
LA English
AB Rhipicephalus sanguineus ticks transmit ***Ehrlichia*** canis, the etiologic agent of canine ***ehrlichiosis*** . In experimentally infected ticks, only ***p30*** -10 transcript was detected among 22 ***p30*** paralogs encoding immunodominant major outer membrane ***P30*** proteins of E. canis. The present study revealed transcription of ***p30*** -10 by E. canis in naturally infected ticks and sequence conservation of ***p30*** -10 genes for E. canis from diverse geographic regions.

L3 ANSWER 9 OF 14 CABA COPYRIGHT 2003 CABI
AN 2002:70420 CABA
DN 20023033646
TI Detection of ***Ehrlichia*** canis in canine carrier blood and in individual experimentally infected ticks with a ***p30*** -based PCR assay
AU Stich, R. W.; ***Rikihisa, Y.*** ; Ewing, S. A.; Needham, G. R.; Grover, D. L.; Jittapalapong, S.
CS Department of Veterinary Preventive Medicine, The Ohio State University, 1900 Coffey Rd., Columbus, OH 43210-1092, USA.
SO Journal of Clinical Microbiology, (2002) Vol. 40, No. 2, pp. 540-546. 37 ref.
ISSN: 0095-1137
DT Journal
LA English
AB Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect ***Ehrlichia*** spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique E. canis gene, ***p30*** , to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for ***Ehrlichia*** chaffeensis p28 were compared to sequences of primers

derived from a sequence conserved among E. canis isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The ***p30*** -based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. chaffeensis, the human granulocytic ***ehrlichiosis*** agent, or ***Ehrlichia*** muris DNA. The assay was used to detect E. canis in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this ***p30*** -based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

L3 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 2002:658778 CAPLUS
DN 137:212016
TI Outer membrane proteins and genes of ***Ehrlichia*** canis and E. chaffeensis and immunochemical methods for diagnosing infections
IN ***Rikihisa, Yasuko*** ; Ohashi, Norio
PA USA
SO U.S. Pat. Appl. Publ., 49 pp., Division of U.S. Ser. No. 314,701.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002120115	A1	20020829	US 2002-59964	20020128
US 6544517	B1	20030408	US 1999-314701	19990519
US 20030103991	US	20030605	US 2002-314639	20021209
PRAI US 1999-314701	A3	19990519		
US 1998-100843P	P	19980918		

AB Diagnostic tools for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans, are provided. The diagnostic tools are a group of outer membrane proteins of E. chaffeensis and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. canis and variants thereof referred to hereinafter as the " ***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. chaffeensis encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The ***P30F*** proteins of E.

canis encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3; ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. chaffeensis OMP proteins and isolated polynucleotides that encode the E. canis ***P30F*** protein are also provided. The present invention also relates to kits contg. reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compns. contg. one or more OMP proteins or ***P30F*** proteins.

L3 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 2001:473743 CAPLUS
DN 138:118175
TI Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** canis and E. chaffeensis. [Erratum to document cited in CA135:340042]
AU Ohashi, Norio; ***Rikihisa, Yasuko*** ; Unver, Ahmet
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SO Infection and Immunity (2001), 69(7), 4702
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB On page 2083, abstr., line 2, "in humans and dogs, resp." should read "in dogs and humans, resp.".

L3 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 1999:219938 CAPLUS
DN 130:249405
TI Outer membrane proteins of ***Ehrlichia*** canis and ***Ehrlichia*** chaffeensis and the genes encoding them and the diagnosis of ***Ehrlichiosis***
IN ***Rikihisa, Yasuko*** ; Ohashi, Noris
PA The Ohio State University, USA
SO PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9913720	A1	19990325	WO 1998-US19600	19980918
W: AU, CA, JP				

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
CA 2304256 AA 19990325 CA 1998-2304256 19980918
AU 9895719 A1 19990405 AU 1998-95719 19980918
AU 748357 B2 20020606
EP 1026949 A1 20000816 EP 1998-949384 19980918
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
PRAI US 1997-59353P P 19970919
WO 1998-US19600 W 19980918
AB Outer membrane proteins of ***Ehrlichia*** that can be used in the serodiagnosis of ***ehrlichiosis*** in man and in Canidae and the genes encoding them are characterized. The genes for a family of OMP-1 proteins of E. chaffeensis and a ***P30*** family of proteins of E. canis are cloned. Sequences of genes and proteins of E. chaffeensis OMP-1, OMP-1A, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, and OMP-1Z are reported. Similarly, sequence of genes and proteins of E. canis ***P30*** , ***P30*** -a, ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, and ***P30*** -10, referred to hereinafter as the ***P30*** family. The present invention also relates to an assay for diagnosing ***ehrlichiosis*** in humans using a recombinant outer membrane protein of E. chaffeensis, particularly OMP-1. The present invention also relates to an assay for diagnosing ***ehrlichiosis*** in humans and members of the family Canidae using a recombinant outer membrane protein of E. canis, particularly ***P30***

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 13 OF 14 LIFESCI COPYRIGHT 2003 CSA
AN 2003:29423 LIFESCI
TI Transcriptional Analysis of ***p30*** Major Outer Membrane Protein Genes of ***Ehrlichia*** canis in Naturally Infected Ticks and Sequence Analysis of ***p30*** - 10 of E. canis from Diverse Geographic Regions
AU Felek, S.; Greene, R.; ***Rikihisa, Y.****
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH 43210-1093; E-mail: rikihisa.1@osu.edu
SO Journal of Clinical Microbiology [J. Clin. Microbiol.], (20020200) vol. 41, no. 2, pp. 886-888.
ISSN: 0095-1137.
DT Journal

FS J
LA English
SL English
AB Rhipicephalus sanguineus ticks transmit ***Ehrlichia*** canis, the etiologic agent of canine ***ehrlichiosis*** . In experimentally infected ticks, only ***p30*** -10 transcript was detected among 22 ***p30*** paralogs encoding immunodominant major outer membrane ***P30*** proteins of E. canis. The present study revealed transcription of ***p30*** -10 by E. canis in naturally infected ticks and sequence conservation of ***p30*** -10 genes for E. canis from diverse geographic regions.

L3 ANSWER 14 OF 14 USPATFULL
AN 2003:152325 USPATFULL
TI Outer membrane protein of ***Ehrlichia*** canis and ***Ehrlichia*** chaffeensis
IN ***Rikihisa, Yasuko*** , Worthington, OH, UNITED STATES
Ohashi, Norio, Columbus, OH, UNITED STATES
PI US 2003103991 A1 20030605
AI US 2002-314639 A1 20021209 (10)
RLI Division of Ser. No. US 1999-314701, filed on 19 May 1999, GRANTED, Pat. No. US 6544517
PRAI US 1998-100853P 19980918 (60)
DT Utility
FS APPLICATION
LREP CALFEE HALTER & GRISWOLD, LLP, 800 SUPERIOR AVENUE, SUITE 1400, CLEVELAND, OH, 44114
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 35 Drawing Page(s)
LN.CNT 4235
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. chaffeensis and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. canis and variants thereof referred to hereinafter as the " ***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. chaffeensis encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The ***P30F*** proteins of E. canis encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9,

P30 -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. chaffeensis OMP proteins and isolated polynucleotides that encode the E. canis ***P30F*** protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.

=> e ohashi n/au
E1 3 OHASHI MUTSUNOBU/AU
E2 1 OHASHI MUTSUO/AU
E3 1179 --> OHASHI N/AU
E4 1 OHASHI NACHITO/AU
E5 2 OHASHI NAGATOSHI/AU
E6 1 OHASHI NAGATSUGU/AU
E7 26 OHASHI NAGAYOSHI/AU
E8 1 OHASHI NAGAYUKI/AU
E9 1 OHASHI NAGOYASHI/AU
E10 3 OHASHI NAHO/AU
E11 10 OHASHI NAME NOT TRANSLATED/AU
E12 1 OHASHI NAMIKO/AU

=> e ohashi norio/au
E1 64 OHASHI NORIKO/AU
E2 7 OHASHI NORIMI/AU
E3 257 --> OHASHI NORIO/AU
E4 1 OHASHI NORIS/AU
E5 1 OHASHI NORITAKA/AU
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E8 48 OHASHI NOZOMI/AU
E9 8 OHASHI NOZOMU/AU
E10 173 OHASHI O/AU
E11 54 OHASHI O M/AU
E12 1 OHASHI O N B/AU

=> s e3 and ehrlich?
L4 45 "OHASHI NORIO"/AU AND EHRLICH?

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 24 DUP REM L4 (21 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 24 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
1
AN 2003:217479 BIOSIS
DN PREV200300217479
TI Outer membrane protein of ***Ehrlichia*** canis and ***Ehrlichia*** chaffeensis.
AU Rikihisa, Yasuko; ***Ohashi, Norio***
ASSIGNEE: The Ohio State University Research Foundation
PI US 6544517 April 08, 2003
SO Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 8 2003) Vol. 1269, No. 2, pp. No Pagination.
http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DT Patent
LA English
AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. chaffeensis and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. canis and variants thereof referred to hereinafter as the "P30F, proteins", and antibodies to the OMP proteins and the P30F proteins. The OMP proteins of E. chaffeensis encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z.
The P3OF proteins of E. canis encompass P30, P30a, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10, P30-11, and P30-12. Isolated polynucleotides that encode the E. chaffeensis OMP proteins and isolated polynucleotides that encode the E. canis P30F protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or P30F proteins.

L5 ANSWER 2 OF 24 USPATFULL
AN 2003:152325 USPATFULL
TI Outer membrane protein of ***Ehrlichia*** canis and ***Ehrlichia*** chaffeensis
IN Rikihisa, Yasuko, Worthington, OH, UNITED STATES
Ohashi, Norio , Columbus, OH, UNITED STATES
PI US 2003103991 A1 20030605
AI US 2002-314639 A1 20021209 (10)

RLI Division of Ser. No. US 1999-314701, filed on 19 May 1999, GRANTED, Pat. No. US 6544517
PRAI US 1998-100853P 19980918 (60)
DT Utility
FS APPLICATION
LREP CALFEE HALTER & GRISWOLD, LLP, 800 SUPERIOR AVENUE, SUITE 1400, CLEVELAND, OH, 44114
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 35 Drawing Page(s)
LN.CNT 4235
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. chaffeensis and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. canis and variants thereof referred to hereinafter as the "P30F proteins", and antibodies to the OMP proteins and the P30F proteins. The OMP proteins of E. chaffeensis encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The P30F proteins of E. canis encompass P30, P30a, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10, P30-11, and P30-12. Isolated polynucleotides that encode the E. chaffeensis OMP proteins and isolated polynucleotides that encode the E. canis P30F protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or P30F proteins.

L5 ANSWER 3 OF 24 USPATFULL
AN 2003:145895 USPATFULL
TI Nucleic acids encoding the major outer membrane protein of the causative agent of human granulocytic ***ehrlichiosis*** and peptides encoded thereby
IN Rikihisa, Yasuko, Worthington, OH, UNITED STATES
Zhi, Ning, Columbus, OH, UNITED STATES
Ohashi, Norio , Columbus, OH, UNITED STATES
PI US 2003099639 A1 20030529
AI US 2002-223598 A1 20020819 (10)
RLI Division of Ser. No. US 1999-288339, filed on 8 Apr 1999, GRANTED, Pat. No. US 6436399
DT Utility
FS APPLICATION

LREP CALFEE HALTER & GRISWOLD, LLP, 800 SUPERIOR AVENUE, SUITE 1400, CLEVELAND, OH, 44114
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 12 Drawing Page(s)
LN.CNT 1341
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to diagnostic tools for serodiagnosing HGE. The diagnostic tools are structurally related proteins, the "Group 44 proteins", and to antibodies to such proteins. the Group 44 proteins comprise a single central hypervariable region of approximately 94 amino acid residues, a first conserved region and a second conserved region which flank the central hypervariable region. The hypervariable region of each Group 44 protein has a higher hydrophilicity, a higher antigenic index, and a higher surface probability than either the first conserved region or the second conserved region of the respective protein. The hypervariable region is basic and has an isoelectric point, i.e., a pI, of from about 7.1 to about 9.2 and a molecular mass, i.e., an Mr, of from about 8.5 kDa to about 11 kDa.. The Group 44 proteins comprise the P44 protein, the P44-2 protein, the P44-12 protein, the P44-15 protein, the P44-18 protein, and the P44-19 protein and variants of such proteins. The present invention also provides isolated polynucleotides or nucleic acids, referred to collectively hereinafter as the "Group 44 polynucleotides", which encode the Group 44 proteins and fragments thereof.. The present invention provides synthetic oligopeptides of 14-16 amino acids in length. Each of the oligopeptides comprise a sequence which is specific to one Group 44 protein. The present invention also relates to antibodies which are immunospecific for and bind to members of the P44 family of proteinss. The present invention also relates to kits containing reagents for diagnosing HGE.

L5 ANSWER 4 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
2
AN 2002:512975 BIOSIS
DN PREV200200512975
TI Nucleic acid encoding the major outer membrane protein of the causative agent of human granulocytic ***ehrlichiosis*** and peptides encoded thereby.
AU Rikihisa, Yasuko; Zhi, Ning (1); ***Ohashi, Norio***
CS (1) Columbus, OH USA
ASSIGNEE: The Ohio State University Research Foundation
PI US 6436399 August 20, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 20, 2002) Vol. 1261, No. 3, pp. No Pagination.

http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DT Patent
LA English
AB The present invention relates to diagnostic tools for serodiagnosing HGE. The diagnostic tools are structurally related proteins, the "Group 44 proteins", and to antibodies to such proteins. the Group 44 proteins comprise a single central hypervariable region of approximately 94 amino acid residues, a first conserved region and a second conserved region which flank the central hypervariable region. The hypervariable region of each Group 44 protein has a higher hydrophilicity, a higher antigenic index, and a higher surface probability than either the first conserved region or the second conserved region of the respective protein. The hypervariable region is basic and has an isoelectric point, i.e., a pI, of from about 7.1 to about 9.2 and a molecular mass, i.e., an Mr, of from about 8.5 kDa to about 11 kDa. The Group 44 proteins comprise the P44 protein, the P44-2 protein, the P44-12 protein, the P44-15 protein, the P44-18 protein, and the P44-19 protein and variants of such proteins. The present invention also provides isolated polynucleotides or nucleic acids, referred to collectively hereinafter as the "Group 44 polynucleotides", which encode the Group 44 proteins and fragments thereof. The present invention provides synthetic oligopeptides of 14-16 amino acids in length. Each of the oligopeptides comprise a sequence which is specific to one Group 44 protein. The present invention also relates to antibodies which are immunospecific for and bind to members of the P44 family of proteinss. The present invention also relates to kits containing reagents for diagnosing HGE.

L5 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
AN 2002:658778 CAPLUS
DN 137:212016
TI Outer membrane proteins and genes of ***Ehrlichia*** canis and E. chaffeensis and immunochemical methods for diagnosing infections
IN Rikihisa, Yasuko; ***Ohashi, Norio***
PA USA
SO U.S. Pat. Appl. Publ., 49 pp., Division of U.S. Ser. No. 314,701.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002120115	A1	20020829	US 2002-59964	20020128
US 6544517	B1	20030408	US 1999-314701	19990519
US 20030103991	US	20030605	US 2002-314639	20021209

PRAI US 1999-314701 A3 19990519
US 1998-100843P P 19980918
AB Diagnostic tools for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans, are provided. The diagnostic tools are a group of outer membrane proteins of E. chaffeensis and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. canis and variants thereof referred to hereinafter as the "P30F proteins", and antibodies to the OMP proteins and the P30F proteins. The OMP proteins of E. chaffeensis encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The P30F proteins of E. canis encompass P30, P30a, P30-1, P30-2, P30-3; P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10, P30-11, and P30-12. Isolated polynucleotides that encode the E. chaffeensis OMP proteins and isolated polynucleotides that encode the E. canis P30F protein are also provided. The present invention also relates to kits contg. reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compns. contg. one or more OMP proteins or P30F proteins.

L5 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 2002:554614 CAPLUS
TI Transcript heterogeneity of the p44 multigene family in a human granulocytic ***ehrlichiosis*** agent transmitted by ticks
AU Zhi, Ning; ***Ohashi, Norio*** ; Tajima, Tomoko; Mott, Jason; Stich, Roger W.; Grover, Debra; Telford, Sam R., III; Lin, Quan; Rikihisa, Yasuko
CS Departments of Veterinary Biosciences and Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SO Infection and Immunity (2002), 70(8), 4754
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal; Errata
LA English
AB Unavailable

L5 ANSWER 7 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
4
AN 2002:460390 BIOSIS
DN PREV200200460390
TI The omp-1 major outer membrane multigene family of ***Ehrlichia*** chaffeensis is differentially expressed in canine and tick hosts.
AU Unver, Ahmet; Rikihisa, Yasuko (1); Stich, Roger W.; ***Ohashi, Norio*** ; Felek, Suleyman

CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd, Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA

SO Infection and Immunity, (August, 2002) Vol. 70, No. 8, pp. 4701-4704. print.
ISSN: 0019-9567.

DT Article

LA English

AB Sixteen of 22 omp-1 paralogs encoding 28-kDa-range immunodominant outer membrane proteins of ***Ehrlichia*** chaffeensis were transcribed in blood monocytes of dogs throughout a 56-day infection period. Only one paralog was transcribed by E. chaffeensis in three developmental stages of Amblyomma americanum ticks before or after E. chaffeensis transmission to naive dogs.

L5 ANSWER 8 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 5

AN 2002:662994 CAPLUS

DN 138:120742

TI Analysis of sequence and loci of p44 homologs expressed by Anaplasma phagocytophila in acutely infected patients

AU Lin, Quan; Zhi, Ning; ***Ohashi, Norio*** ; Horowitz, Harold W.; Aguero-Rosenfeld, Maria E.; Raffalli, John; Wormser, Gary P.; Rikihisa, Yasuko

CS Dep. of Vet. Biosci., Coll. of Vet. Med., The Ohio State Univ., Columbus, OH, 43210, USA

SO Journal of Clinical Microbiology (2002), 40(8), 2981-2988
CODEN: JCMIDW; ISSN: 0095-1137

PB American Society for Microbiology

DT Journal

LA English

AB Anaplasma phagocytophila is an obligatory intragranulocytic bacterium that causes human granulocytic ***ehrlichiosis*** . Immunodominant 44-kDa outer membrane proteins of A. phagocytophila are encoded by a p44 multigene family. The expression profiles of p44 genes in the blood of acutely infected patients in the year 2000 were characterized. A single p44 gene was predominantly expressed in peripheral blood leukocytes from one patient, while up to 17 different p44 genes were transcribed without a single majority in the other two patients. The cDNA sequences of the central hypervariable region of several p44 genes were identical among the isolated from the three patients and a 1995 A. phagocytophila isolate. A. phagocytophila was isolated by cell culture from all of the three 2000 patients. Genomic Southern blot anal. of the three 2000 and two 1995 A. phagocytophila with probes specific to the most dominant p44 transcript in each patient showed that the p44 loci in the A. phagocytophila genome were conserved. Anal. of the predicted amino acid sequences of 43 different

p44 genes including 19 new sequences found in the present study, revealed that five amino acids were absolutely conserved. The hypervariable region was subdivided into five domains, including three extremely hypervariable central domains. The results suggest that variations in the sequences of p44 are not random but are restricted. Furthermore, several p44 genes are not hypermutatable in nature, based on the conservation of gene sequences and loci among isolates obtained 5 yr apart.

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC.DUPLICATE
6

AN 2002:255482 BIOSIS

DN PREV200200255482

TI Characterization and transcriptional analysis of gene clusters for a type IV secretion machinery in human granulocytic and monocytic ***ehrlichiosis*** agents.

AU ***Ohashi, Norio*** ; Zhi, Ning; Lin, Quan; Rikihisa, Yasuko (1)

CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA

SO Infection and Immunity, (April, 2002) Vol. 70, No. 4, pp. 2128-2138. print.
ISSN: 0019-9567.

DT Article

LA English

AB Anaplasma (***Ehrlichia***) phagocytophila and ***Ehrlichia*** chaffeensis, the etiologic agents of granulocytic and monocytic ***ehrlichioses*** , respectively, are obligatory intracellular bacteria that cause febrile systemic illness in humans. We identified and characterized clusters of genes for a type IV secretion machinery in these two bacteria, and analyzed their gene expression in cell culture and mammalian hosts. Eight virB and virD genes were found in each bacterial genome, and all of the genes were transcribed in cell culture. Although the gene order and orientation were similar to those found in other bacteria, the eight virB and virD genes were clustered at two separate loci in each genome. Five of the genes (virB8, virB9, virB10, virB11, and virD4) were located downstream from a ribA gene. These five genes in both A. phagocytophila and E. chaffeensis were polycistronically transcribed and controlled through at least two tandem promoters located upstream of the virB8 gene in human leukemia cell lines. The virB9 gene of A. phagocytophila was transcriptionally active in peripheral blood leukocytes from human ***ehrlichiosis*** patients and experimentally infected animals. Three of the remaining genes (virB3, virB4, and virB6) of both A.

phagocytophila and E. chaffeensis were arranged downstream from a sodB gene and cotranscribed with the sodB gene through one or more sodB promoters in human leukocytes. This suggests that transcription of the three virB genes in these two Anaplasma and ***Ehrlichia*** spp. is regulated by factors that influence the sodB gene expression. This unique regulation of gene expression for the type IV secretion system may be associated with intracellular survival and replication of Anaplasma and ***Ehrlichia*** spp. in granulocytes or monocytes.

L5 ANSWER 10 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
7
AN 2002:197226 BIOSIS
DN PREV200200197226
TI Transcript heterogeneity of the p44 multigene family in a human granulocytic ***ehrlichiosis*** agent transmitted by ticks.
AU Zhi, Ning; ***Ohashi, Norio*** ; Tajima, Tomoko; Mott, Jason; Stich, Roger W.; Grover, Debra; Telford, Sam R., III; Lin, Quan; Rikihisa, Yasuko (1)
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA
SO Infection and Immunity, (March, 2002) Vol. 70, No. 3, pp. 1175-1184. print.
ISSN: 0019-9567.
DT Article
LA English
AB Human granulocytic ***ehrlichiosis*** (HGE) is an emerging tick-borne zoonosis caused by a strain of Anaplasma phagocytophila called the HGE agent, an obligatory intracellular bacterium. The agent expresses immunodominant 44-kDa outer membrane proteins (P44s) encoded by a multigene family. The present study established an experimental process for transmission of the HGE agent from infected mice (a reservoir model) to nymphal Ixodes scapularis ticks (a biological vector) and subsequently to horses (a patient model) by the adult infected ticks. Overall, a total of 20 different p44 transcripts were detected in the mammals, ticks, and cell cultures. Among them, a transcript from a p44-18 gene was major at acute stage in mice and horses but minor in ticks. Both mRNA and protein produced from the p44-18 gene were detected in the HGE agent cultivated in HL-60 cells at 37degreeC, but their expression levels decreased in the organisms cultivated at 24degreeC, suggesting that temperature is one of the factors that influence the expression of members of the p44 multigene family. Several additional p44 transcripts that were not detected in the mammals at the acute stage of infection were detected in ticks. Phylogenetic analysis of the 20 different p44 transcripts revealed that

the major transcripts found in mammals and ticks were distinct, suggesting a difference in surface propreties between populations of the HGE agent in different host environments. The present study provides new information for understanding the role of the p44 multigene family in transmission of the HGE agent between mammals and ticks.

L5 ANSWER 11 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
8
AN 2002:564818 BIOSIS
DN PREV200200564818
TI Activation of a p44 pseudogene in Anaplasma phagocytophila by bacterial RNA splicing: A novel mechanism for post-transcriptional regulation of a multigene family encoding immunodominant major outer membrane proteins.
AU Zhi, Ning; ***Ohashi, Norio*** ; Rikihisa, Yasuko (1)
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Road, Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA
SO Molecular Microbiology, (October, 2002) Vol. 46, No. 1, pp. 135-145. http://www.mol.micro.com. print.
ISSN: 0950-382X.
DT Article
LA English
AB Immunodominant 44 kDa major outer membrane proteins of Anaplasma phagocytophila (human granulocytic ***ehrlichiosis*** agent) are encoded by the p44 multigene family. One of the paralogues, p44-18 is predominantly expressed by A. phagocytophila in mammalian hosts, but is downregulated in the arthropod vector. The expression of p44-18 was upregulated in A. phagocytophila cultivated in HL-60 cells at 37degreeC compared with 24degreeC. However, the molecular mechanism of such gene expression was unclear, as p44-18 has a pseudogene-like structure, i.e. it lacks an AUG start codon and is out of frame with an upstream overlapping paralogue, p44-1. In the present study, we found that an amplicon detected by reverse transciption-polymerase chain reaction (RT-PCR) (808 basepair (bp)) for the p44-1/p44-18 gene locus was smaller than that detected by PCR with the genomic DNA (1652 bp) in the A. phagocytophila-infected HL-60 cells cultured at 37degreeC. A circularized RNA molecule corresponding to the 844 bp region missing from the locus in the RT-PCR product was detected by inverse RT-PCR, indicating that this is an intron (designated p44-1 intervening sequence, p44-1 IVS). The splicing event of p44-1 IVS was also observed when the p44-1 IVS-carrying plasmid was introduced into Escherichia coli, suggesting that the splicing is sequence-dependent. Structural analysis and in vitro splicing experiments of p44-1 IVS suggested that this is likely to represent a new class of introns in eubacteria. The primer extension analysis showed the presence of a

putative sigma32-type promoter in region upstream from p44-1. Collectively, the novel RNA splicing and the temperature-dependent transcription may account for the dominant p44-18 expression in mammals.

L5 ANSWER 12 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
9
AN 2001:504077 BIOSIS
DN PREV200100504077
TI Transcriptional analysis of p30 major outer membrane multigene family of ***Ehrlichia*** canis in dogs, ticks, and cell culture at different temperatures.
AU Unver, Ahmet; ***Ohashi, Norio*** ; Tajima, Tomoko; Stich, Roger W.; Grover, Debra; Rikihisa, Yasuko (1)
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA
SO Infection and Immunity, (October, 2001) Vol. 69, No. 10, pp. 6172-6178. print.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB ***Ehrlichia*** canis, an obligatory intracellular bacterium of monocytes and macrophages, causes canine monocytic ***ehrlichiosis*** . E. canis immunodominant 30-kDa major outer membrane proteins are encoded by a polymorphic multigene family consisting of more than 20 paralogs. In the present study, we analyzed the mRNA expression of 14 paralogs in experimentally infected dogs and Rhipicephalus sanguineus ticks by reverse transcription-PCR using gene-specific primers followed by Southern blotting. Eleven out of 14 paralogs in E. canis were transcribed in increasing numbers and transcription levels, while the mRNA expression of the 3 remaining paralogs was not detected in blood monocytes of infected dogs during the 56-day postinoculation period. Three different groups of R. sanguineus ticks (adult males and females and nymphs) were separately infected with E. canis by feeding on the infected dogs. In these pools of acquisition-fed ticks as well as in the transmission-fed adult ticks, the transcript from only one paralog was detected, suggesting the predominant transcription of that paralog or the suppression of the remaining paralogs in ticks. Expression of the same paralog was higher whereas expression of the remaining paralogs was lower in E. canis cultivated in dog monocyte cell line DH82 at 25degreeC than in E. canis cultivated at 37degreeC. Analysis of differential expression of p30 multigenes in dogs, ticks, or monocyte cell cultures would help in understanding the role of these gene products in pathogenesis and E. canis transmission as well as in designing

a rational vaccine candidate immunogenic against canine ***ehrlichiosis*** .

L5 ANSWER 13 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:310619 BIOSIS
DN PREV200200310619
TI (Correction of Previews 200100211740. Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** canis and E. chaffeensis. Correction of abstract.
AU ***Ohashi, Norio*** ; Rikihisa, Yasuko (1); Unver, Ahmet
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa@osu.edu USA
SO Infection and Immunity, (July, 2001) Vol. 69, No. 7, pp. 4702. print. ISSN: 0019-9567.
DT Article
LA English
AB Volume 69, no. 4, p. 2083-2091, 2001. Page 2083, abstract, line 2: "in humans and dogs, respectively" should read "in dogs and humans, respectively".

L5 ANSWER 14 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 2001:473743 CAPLUS
DN 138:118175
TI Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** canis and E. chaffeensis. [Erratum to document cited in CA135:340042]
AU ***Ohashi, Norio*** ; Rikihisa, Yasuko; Unver, Ahmet
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SO Infection and Immunity (2001), 69(7), 4702
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB On page 2083, abstr., line 2, "in humans and dogs, resp." should read "in dogs and humans, resp.".

L5 ANSWER 15 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 2001:211740 BIOSIS
DN PREV200100211740
TI Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** canis and E. chaffeensis.

AU ***Ohashi, Norio*** ; Rikihisa, Yasuko (1); Unver, Ahmet
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa@osu.edu USA
SO Infection and Immunity, (April, 2001) Vol. 69, No. 4, pp. 2083-2091. print.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB ***Ehrlichia*** canis and E. chaffeensis are tick-borne obligatory intramonocytic ***ehrlichiae*** that cause febrile systemic illness in humans and dogs, respectively. The current study analyzed the pleomorphic multigene family encoding approximately 30-kDa major outer membrane proteins (OMPs) of E. canis and E. chaffeensis. Upstream from secA and downstream of hypothetical transcriptional regulator, 22 paralogs of the omp gene family were found to be tandemly arranged except for one or two genes with opposite orientations in a 28- and a 27-kb locus in the E. canis and E. chaffeensis genomes, respectively. Each locus consisted of three highly repetitive regions with four nonrepetitive intervening regions. E. canis, in addition, had a 6.9-kb locus which contained a repeat of three tandem paralogs in the 28-kb locus. These total 47 paralogous and orthologous genes encoded OMPs of approximately 30 to 35 kDa consisting of several hypervariable regions alternating with conserved regions. In the 5'-end half of the 27-kb locus or the 28-kb locus of each ***Ehrlichia*** species, 14 paralogs were linked by short intergenic spaces ranging from -8 bp (overlapped) to 27 bp, and 8 remaining paralogs in the 3'-end half were connected by longer intergenic spaces ranging from 213 to 632 bp. All 22 paralogs, five unknown genes, and secA in the omp cluster in E. canis were transcriptionally active in the monocyte culture, and the paralogs with short intergenic spaces were cotranscribed with their adjacent genes, including the respective intergenic spaces at both the 5' and the 3' sides. Although omp genes are diverse, our results suggest that the gene organization of the clusters and the gene locus are conserved between two species of ***Ehrlichia*** to maintain a unique transcriptional mechanism for adaptation to environmental changes common to them.

L5 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
11
AN 2000:455720 BIOSIS
DN PREV200000455720
TI Analysis of 16S rRNA and 51-kilodalton antigen gene and transmission in mice of ***Ehrlichia*** risticii in virgulate trematodes from Elimia

livescens snails in Ohio.

AU Kanter, Manuel; Mott, Jason; ***Ohashi, Norio*** ; Fried, Bernard; Reed, Stephen; Lin, Young C.; Rikihisa, Yasuko (1)

CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093 USA

SO Journal of Clinical Microbiology, (September, 2000) Vol. 38, No. 9, pp. 3349-3358. print.
ISSN: 0095-1137.

DT Article

LA English

SL English

AB Operculate snails (the family Pleuroceridae: Elimia livescens) were collected between June and October 1998 from a river in central Ohio where repeated cases of Potomac horse fever (PHF) have occurred. Of collected snails, consistently 50 to 80% carried a combination of cercariae and sporocysts of digenetic virgulate trematodes. The trematodes obtained from each snail were pooled and tested for ***Ehrlichia*** risticii, the agent of PHF, by nested PCR using primers specific to the 16S rRNA gene. Out of a total of 209 trematode pools, 50 pools were found to be positive by PCR. The DNA sequence of the 16S rRNA gene identified in one trematode pool was identical to that of the type strain of E. risticii, and the sequence of the gene identified in another pool differed from that of the type strain by 1 nucleotide. Comparison of the deduced amino acid sequence of the partial 51-kDa antigen gene from various sources revealed that Maryland, Ohio (except Ohio 081), and Kentucky strains are in a cluster distinct from the sequences obtained from sources in California and Oregon. Ohio 081 was shown previously by antigenic composition analysis to be distinct from other groups. However, all sequences examined were not segregated according to their sources: horse blood or infected trematodes. E. risticii was found to be transmittable from trematodes to mice and was subsequently passaged from infected mice to additional mice, as determined by PCR analysis. Our findings suggest the evolution of E. risticii in the natural reservoir in separate geographic regions and persistent infection of trematode populations with E. risticii during summer and early fall. The study also suggests that the mouse can be used to isolate E. risticii from the infected trematode.

L5 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2003 ACS

AN 1999:672518 CAPLUS

DN 131:307683

TI Nucleic acids encoding outer membrane protein of human granulocytic ***ehrlichiosis*** agent

IN Rikhisa, Yasuko; Zhi, Ning; ***Ohashi, Norio***

PA The Ohio State Research Foundation, USA

SO PCT Int. Appl., 52 pp.

CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9952370	A1	19991021	WO 1999-US7759	19990408
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2326717	AA	19991021	CA 1999-2326717	19990408
AU 9934835	A1	19991101	AU 1999-34835	19990408
EP 1069827	A1	20010124	EP 1999-916535	19990408
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
US 6436399	B1	20020820	US 1999-288339	19990408
US 2003099639	A1	20030529	US 2002-223598	20020819

PRAI US 1998-81192P P 19980409
US 1999-128087P P 19990407
US 1999-288339 A3 19990408
WO 1999-US7759 W 19990408

AB The invention provides tools for serodiagnosing human granulocytic ***ehrlichiosis*** (HGE). The tools are structurally related proteins, "Group 44 proteins", and antibodies to such proteins. The Group 44 proteins comprise a single central hypervariable region, a first conserved region and a second conserved region which flank the central hypervariable region. The Group 44 proteins comprise P44, P44-2, P44-12, P44-15, P44-18, P44-19, and variants of such proteins. The invention also provides isolated polynucleotides or nucleic acids, referred to as "Group 44 polynucleotides", which encode the Group 44 proteins and fragments thereof. Each oligopeptide comprises a sequence specific to one Group 44 protein. The present invention also provides antibodies immunospecific for and which bind to members of the P44 family of proteins. The present invention provides kits contg. reagent for diagnosing HGE.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 18 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
12
AN 1999:342215 BIOSIS
DN PREV199900342215
TI Multiple p44 genes encoding major outer membrane proteins are expressed in the human granulocytic ***ehrlichiosis*** agent.
AU Zhi, Ning; ***Ohashi, Norio*** ; Rikihisa, Yasuko (1)
CS (1) Dept. of Veterinary Biosciences, College of Veterinary Medicine, Ohio

State University, 1925 Coffey Rd., Columbus, OH, 43210-1093 USA
SO Journal of Biological Chemistry, (June 18, 1999) Vol. 274, No. 25, pp. 17828-17836.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB Human granulocytic ***ehrlichiosis*** (HGE) is caused by infection with an obligatory intracellular bacterium, the HGE agent. We previously cloned a gene encoding HGE agent 44-kDa major outer membrane protein and designated it p44. In this study, we (i) identified five different mRNAs that are transcribed from p44-homologous genes in the HGE agent cultivated in HL-60 cells; (ii) cloned genes corresponding to the mRNAs from the genomic DNA of the HGE agent; (iii) showed that the genes being expressed were not clustered in the HGE agent genome; (iv) estimated that a minimum copy number of the p44-homologous genes in the genome is 18; (v) detected two different P44-homologous proteins expressed by the HGE agent; and (vi) demonstrated existence of antibodies specific to the two proteins in sera from patients with HGE. These findings showed that p44 multigenes have several active expression sites and the expression is regulated at transcriptional level, suggesting a potentially unique mechanism for generating the diversity in major antigenic outer membrane proteins of the HGE agent. Characterization of p44-homologous genes expressed by the HGE agent in a tissue culture would assist in understanding a role of the p44 multigene family in pathogenesis and immune response in HGE.

L5 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 1999:809483 CAPLUS
DN 132:277873
TI Western and dot blotting analyses of ***Ehrlichia*** chaffeensis indirect fluorescent-antibody assay-positive and -negative human sera by using native and recombinant E. chaffeensis and E. canis antigens
AU Unver, Ahmet; Rikihisa, Yasuko; ***Ohashi, Norio*** ; Cullman, Louis C.; Buller, Richard; Storch, Gregory A.
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SO Journal of Clinical Microbiology (1999), 37(12), 3888-3895
CODEN: JCMIDW; ISSN: 0095-1137
PB American Society for Microbiology
DT Journal
LA English
AB Human monocytic ***ehrlichiosis*** is an emerging infectious disease caused by ***Ehrlichia*** chaffeensis, a gram-neg. obligatory intracellular bacterium closely related to E. canis. The immunoreactive recombinant fusion proteins rP28 and rP30 have become available after

cloning and expressing of the 28- and 30-kDa major outer membrane protein genes of E. chaffeensis and E. canis, resp. Western immunoblotting was performed to analyze the antibody responses of the 37 E. chaffeensis indirect fluorescent-antibody assay (IFA)-pos. and 20 IFA-neg. serum specimens with purified whole organisms, rP28, and rP30. All IFA-neg. sera were neg. with purified whole organisms, rP28, or rP30 by Western immunoblot anal. (100% relative diagnostic specificity). Of 37 IFA-pos. sera, 34 sera reacted with any native proteins of E. chaffeensis ranging from 44 to 110 kDa, and 30 sera reacted with 44- to 110-kDa native E. canis antigens. The 28-kDa E. chaffeensis and 30-kDa E. canis native proteins were recognized by 25 IFA-pos. sera. Fifteen IFA-pos. sera reacted with rP28 by Western blot anal., whereas 34 IFA-pos. sera reacted with rP30 (92% relative diagnostic specificity), indicating that rP30 is more sensitive than rP28 for detecting the antibodies in IFA-pos. sera. These 34 IFA-pos. sera were pos. by the dot blot assay with rP30, distinguishing them from IFA-neg. sera. Except for three rP30-neg. but IFA-pos. specimens that instead showed an E. ewingii infection-like profile by Western immunoblotting, the results of Western and dot blot assays with rP30 matched 100% with the IFA test results. Densitometric anal. of dot blot reactions showed a pos. correlation between the dot d. and the IFA titer. These results suggest that rP30 antigen would provide a simple, consistent, and rapid serodiagnosis for human monocytic ***ehrlichiosis*** .

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 20 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
13

AN 1999:262685 BIOSIS

DN PREV199900262685

TI ***Ehrlichia*** chaffeensis and E. sennetsu, but not the human granulocytic ***ehrlichiosis*** agent, colocalize with transferrin receptor and up-regulate transferrin receptor mRNA by activating iron-responsive protein 1.

AU Barnewall, Roy E.; ***Ohashi, Norio*** ; Rikihisa, Yasuko (1)

CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1092 USA

SO Infection and Immunity, (May, 1999) Vol. 67, No. 5, pp. 2258-2265. ISSN: 0019-9567.

DT Article

LA English

SL English

AB ***Ehrlichia*** chaffeensis and E. sennetsu are genetically divergent obligatory intracellular bacteria of human monocytes and macrophages, and

the human granulocytic ***ehrlichiosis*** (HGE) agent is an obligatory intracellular bacterium of granulocytes. Infection with both E. chaffeensis and E. sennetsu, but not HGE agent, in the acute monocytic leukemia cell line THP-1 almost completely inhibited by treatment with deferoxamine, a cell-permeable iron chelator. Transferrin receptors (TfRs) accumulated on both E. chaffeensis and E. sennetsu, but not HGE agent, inclusions in THP-1 cells or the cells of the promyelocytic leukemia cell line HL-60. Reverse transcription-PCR showed an increase in the level of TfR mRNA 6 h postinfection which peaked at 24 h postinfection with both E. chaffeensis and E. sennetsu infection in THP-1 or HL-60 cells. In contrast, HGE agent in THP-1 or HL-60 cells induced no increase in TfR mRNA levels. Heat treatment of E. chaffeensis or the addition of monodansylcadaverine, a transglutaminase inhibitor, 3 h prior to infection inhibited the up-regulation of TfR mRNA. The addition of oxytetracycline 6 h after E. chaffeensis infection caused a decrease in TfR mRNA which returned to the basal level by 24 h postinfection. These results indicate that both internalization and continuous proliferation of ***ehrlichial*** organisms or the production of ***ehrlichial*** proteins are required for the up-regulation of TfR mRNA. Results of electrophoretic mobility shift assays showed that both E. chaffeensis and E. sennetsu infection increased the binding activity of iron-responsive protein 1 (IRP-1) to the iron-responsive element at 6 h postinfection and remained elevated at 24 h postinfection. However, HGE agent infection had no effect on IRP-1 binding activity. This result suggests that activation of IRP-1 and subsequent stabilization of TfR mRNA comprise the mechanism of TfR mRNA up-regulation by E. chaffeensis and E. sennetsu infection.

L5 ANSWER 21 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14

AN 1998:348478 BIOSIS

DN PREV199800348478

TI Cloning of the heat shock protein 70 (HSP70) gene of ***Ehrlichia*** sennetsu and differential expression of HSP70 and HSP60 mRNA after temperature upshift.

AU Zhang, Yilan; ***Ohashi, Norio*** ; Rikhisa, Yasuko (1)

CS (1) Dep. Vet. Biosci., Coll. Vet. Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210 USA

SO Infection and Immunity, (July, 1998) Vol. 66, No. 7, pp. 3106-3112. ISSN: 0019-9567.

DT Article

LA English

AB ***Ehrlichia*** sennetsu is the causative agent of human Sennetsu ***ehrlichiosis*** . Heat shock protein 60 (HSP60) and HSP70 (DnaK) are two major bacterial HSPs, and their interaction modulates the stress

response. Previously, we cloned and sequenced groE and expressed groEL of E. sennetsu. HSP60 (GroEL) was immunogenic and cross-reactive in ***Ehrlichia*** spp. The present study was designed to (i) characterize the HSP70 gene of this organism and (ii) determine whether the expression of these two HSPs is inducible upon exposure to heat stress. A gene encoding an HSP70 homolog was isolated and sequenced from a gene library. The ***ehrlichial*** HSP70 gene encoded a 637-amino-acid protein, which had an approximate molecular mass of 68,354 Da and which was homologous to DnaK of Escherichia coli. A DNA sequence resembling -35 and -10 promoter sequences of E. coli dnaK was observed upstream of the ***ehrlichial*** HSP70 gene. Alignment of the predicted amino acid sequence with that of E. coli DnaK and Brucella, Salmonella, Borrelia, Chlamydia, and Mycobacterium HSP70s showed 63, 67, 63, 62, 58, and 53% identity, respectively. By reverse transcription-PCR analysis, the mRNA levels of ***ehrlichial*** HSP70 and HSP60 were examined after temperature shifts from 28 to 37degree C and from 37 to 40degree C. HSP70 mRNA induction levels were greater than those of HSP60 mRNA after a 37-to-40degree C temperature shift, whereas the reverse was true after a 28-to-37degree C temperature shift. Our data suggest that HSP60 and HSP70 may play different roles during transfer from vector temperature to human body temperature and during a febrile condition characteristic of ***ehrlichial*** disease. This study also provides a useful model system for examining mRNA expression in obligatory intracellular bacteria.

L5 ANSWER 22 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
15
AN 1998:435123 BIOSIS
DN PREV199800435123
TI Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of ***Ehrlichia*** canis and application of the recombinant protein for serodiagnosis.
AU ***Ohashi, Norio*** ; Unver, Ahmet; Zhi, Ning; Rikihisa, Yasuko (1)
CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Journal of Clinical Microbiology, (Sept., 1998) Vol. 36, No. 9, pp. 2671-2680.
ISSN: 0095-1137.
DT Article
LA English
AB A 30-kDa major outer membrane protein of ***Ehrlichia*** canis, the agent of canine ***ehrlichiosis*** , is the major antigen recognized by both naturally and experimentally infected dog sera. The protein cross-reacts with a serum against a recombinant 28-kDa protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of

Ehrlichia chaffeensis. Two DNA fragments of E. canis were amplified by PCR with two primer pairs based on the sequences of E. chaffeensis omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of E. canis. Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E. canis genome. Three copies of the entire gene (p30, p30-1, and p30a) were cloned and sequenced from the E. canis genomic DNA. The open reading frames of the two copies (p30 and p30-1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E. canis 30-kDa protein genes and the E. chaffeensis omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp., p44 from the agent of human granulocytic ***ehrlichiosis*** , msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. canis 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E. canis 30-kDa proteins are closely related but that the third 30-kDa protein is not. The p30 gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified E. canis. Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 of E. canis. To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ***ehrlichiosis*** , the immunoreactions between rP30 and the whole purified E. canis antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IFA-positive and -negative plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ***ehrlichiosis*** . Cloning of multigenes encoding the 30-kDa major outer membrane proteins of E. canis will greatly facilitate understanding pathogenesis and immunologic study of canine ***ehrlichosis*** and provide a useful tool for phylogenetic analysis.

L5 ANSWER 23 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
16
AN 1998:76405 BIOSIS
DN PREV199800076405
TI Immunodominant major outer membrane proteins of ***Ehrlichia*** chaffeensis are encoded by a polymorphic multigene family.
AU ***Ohashi, Norio*** ; Zhi, Ning; Zhang, Yilan; Rikihisa, Yasuko (1)

CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Infection and Immunity, (Jan., 1998) Vol. 66, No. 1, pp. 132-139. ISSN: 0019-9567.
DT Article
LA English
AB Several immunodominant major proteins ranging from 23 to 30 kDa were identified in the outer membrane fractions of ***Ehrlichia*** chaffeensis and ***Ehrlichia*** canis. The N-terminal amino acid sequence of a 28-kDa protein of E. chaffeensis (one of the major proteins) was determined. The gene (p28), almost full length, encoding the 28-kDa protein was cloned by PCR with primers designed based on the N-terminal sequence of the E. chaffeensis 28-kDa protein and the consensus sequence between the C termini of the Cowdria ruminantium MAP-1 and Anaplasma marginale MSP-4 proteins. The p28 gene was overexpressed, and antibody to the recombinant protein was raised in a rabbit. The antibody and serum from a patient infected with E. chaffeensis reacted with the recombinant protein, three proteins (29, 28, and 25 kDa) of E. chaffeensis, and a 30-kDa protein of E. canis. Immunoelectron microscopy with the rabbit antibody revealed that the antigenic epitope of the 28-kDa protein was exposed on the surface of E. chaffeensis. Southern blot analysis with a 32P-labeled p28 gene probe revealed multiple copies of genes homologous to p28 in the E. chaffeensis genome. Six copies of the p28 gene were cloned and sequenced from the genomic DNA by using the same probe. The open reading frames of these gene copies were tandemly arranged with intergenic spaces. They were nonidentical genes and contained a semivariable region and three hypervariable regions in the predicted protein molecules. One of the gene copies encoded a protein with an internal amino acid sequence identical to the chemically determined N-terminal amino acid sequence of a 23-kDa protein of E. chaffeensis. Immunization with the recombinant P28 protein protected mice from infection with E. chaffeensis. These findings suggest that the 30-kDa-range proteins of E. chaffeensis represent a family of antigenically related homologous proteins encoded by a single gene family.

L5 ANSWER 24 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 17
AN 1997:366899 BIOSIS
DN PREV199799658832
TI ***Ehrlichia*** sennetsu groE operon and antigenic properties of the GroEL homolog.
AU Zhang, Yilan; ***Ohashi, Norio*** ; Lee, Eunjoo H.; Tamura, Akira; Rikihisa, Yasuko (1)
CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ.,

1925 Coffey Road, Columbus, OH 43210 USA
SO FEMS Immunology and Medical Microbiology, (1997) Vol. 18, No. 1, pp. 39-46.
ISSN: 0928-8244.
DT Article
LA English
AB A clone expressing an immunoreactive 55-kilodalton (kDa) protein of ***Ehrlichia*** sennetsu, the causative agent of human Sennetsu ***ehrlichiosis*** , was isolated from a gene library of this organism. Sequence analysis of the DNA insert revealed two open reading frames, encoding proteins of 10,620 and 58,225 kDa, respectively. These deduced amino acid sequences were homologous to those of the GroES and GroEL heat shock proteins (HSP) of other bacteria, respectively. Phylogenetic trees based on GroES and GroEL homologs of several bacteria including E. sennetsu showed a relationship similar to that based on 16S rRNA gene sequences. The recombinant and native 55-kDa proteins of E. sennetsu, GroEL homolog, reacted with a monoclonal antibody (SPA807) which recognizes a homologous sequence between human and mycobacterial HSP60 and a polyclonal antibody (SPA804) to cyanobacteria HSP60, but not with antibodies to HSP60 of several other organisms used. Furthermore, anti-recombinant E. sennetsu 55-kDa protein antibody prepared in a rabbit was reactive to HSP60 antigens of other ***Ehrlichia*** and Rickettsia species, but not GroEL of E. coli. The recombinant 55-kDa protein would be a useful tool for studying the role of this antigen in the immune response to E. sennetsu infection.

=> s ehrlich? and (chaffeensis or canis) and p30?
L6 61 EHRLICH? AND (CHAFFEENSIS OR CANIS) AND P30?

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 30 DUP REM L6 (31 DUPLICATES REMOVED)

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 30 ANSWERS - CONTINUE? Y/(N):y

L7 ANSWER 1 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
1
AN 2003:217479 BIOSIS
DN PREV200300217479
TI Outer membrane protein of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** .
AU Rikihisa, Yasuko; Ohashi, Norio

ASSIGNEE: The Ohio State University Research Foundation
PI US 6544517 April 08, 2003
SO Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 8 2003) Vol. 1269, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
DT Patent
LA English
AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the " ***P30F*** , proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The P3OF proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. ***chaffeensis*** OMP proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.
TI Outer membrane protein of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** .
AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the " ***P30F*** , proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The P3OF proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. ***chaffeensis*** OMP

proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.

IT Major Concepts
Biochemistry and Molecular Biophysics; Human Medicine (Medical Sciences); Infection; Methods and Techniques; Veterinary Medicine (Medical Sciences)

IT Diseases
ehrlichiosis : bacterial disease, diagnosis

IT Chemicals & Biochemicals
Ehrlichia ***canis*** outer membrane proteins; ***Ehrlichia*** ***chaffeensis*** outer membrane proteins

IT Alternate Indexing
Ehrlichiosis (MeSH)

ORGN Super Taxa
Canidae: Carnivora, Mammalia, Vertebrata, Chordata, Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Ehrlichia ***canis*** (Rickettsiaceae): pathogen; ***Ehrlichia*** ***chaffeensis*** (Rickettsiaceae): pathogen; canid (Canidae): host

ORGN Organism Superterms
Animals; Bacteria; Carnivores; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Vertebrates

L7 ANSWER 2 OF 30 USPATFULL

AN 2003:152325 USPATFULL

TI Outer membrane protein of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***

IN Rikihisa, Yasuko, Worthington, OH, UNITED STATES
Ohashi, Norio, Columbus, OH, UNITED STATES

PI US 2003103991 A1 20030605

AI US 2002-314639 A1 20021209 (10)

RLI Division of Ser. No. US 1999-314701, filed on 19 May 1999, GRANTED, Pat. No. US 6544517

PRAI US 1998-100853P 19980918 (60)

DT Utility

FS APPLICATION

LREP CALFEE HALTER & GRISWOLD, LLP, 800 SUPERIOR AVENUE, SUITE 1400, CLEVELAND, OH, 44114

CLMN Number of Claims: 23

ECL Exemplary Claim: 1

DRWN 35 Drawing Page(s)
LN.CNT 4235
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the " ***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The ***P30F*** proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. ***chaffeensis*** OMP proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.

TI Outer membrane protein of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***

AB Diagnostic tools for for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans are provided. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the " ***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The ***P30F*** proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. ***chaffeensis*** OMP proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** protein are also provided. The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and

canine ***ehrlichiosis*** , and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.

SUMM [0002] The ***ehrlichiae*** are obligate intracellular bacteria that infect circulating leucocytes. ***Ehrlichia*** ***chaffeensis*** infects the monocytes and macrophages in humans and causes human monocytic ***ehrlichiosis*** . The clinical manifestations of ***ehrlichiosis*** in humans are nonspecific and similar to Rocky Mountain spotted fever. The clinical manifestations include fever, chills, headache, myalgia or. . .

SUMM [0003] ***Ehrlichia*** ***canis*** infects and causes ***ehrlichiosis*** in animals belonging to the family Canidae. Canine ***ehrlichiosis*** consists of an acute and a chronic phase. The acute phase is characterized by fever, serous nasal and ocular discharges,. . . However, chronically infected dogs do not, respond well to the antibiotic. Therefore, early diagnosis is very important for treating canine ***ehrlichiosis*** .

SUMM [0004] The primary diagnostic test for diagnosing canine ***ehrlichiosis*** and human ***ehrlichiosis*** is the indirect fluorescent antibody (IFA) test. This test uses the etiologic agent ***Ehrlichia*** ***canis*** to diagnose canine ***ehrlichiosis*** . The IFA test uses ***Ehrlichia*** ***chaffeensis*** as antigen for diagnosing human ***ehrlichiosis*** . The IFA test has, however, serious limitations. The IFA test is subject to false positives because the antigens are made. . .

SUMM [0005] Tools which permit simpler, more rapid, and objective serodiagnosis of canine ***ehrlichiosis*** or human ***ehrlichiosis*** are desirable.

SUMM [0006] The present invention relates to improved diagnostic tools for veterinary and human use which are used for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the "***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins.

SUMM [0007] The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The mature OMP-1 protein of E. ***chaffeensis*** has a molecular weight of about 27.7 kDa and comprises amino acid 26 through amino acid 281 of the sequence shown in FIG. 3B, SEQ ID NO: 2. The mature OMP-1B protein of E. ***chaffeensis*** has a molecular weight of about 28.2 kDa and comprises amino acid 26 through amino acid 283 of the sequence shown in FIG. 4B, SEQ ID NO: 4. The mature OMP-1C protein of E.

chaffeensis has a molecular weight of about 27.6 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 5B, SEQ ID NO: 6. The mature OMP-1D protein of E. ***chaffeensis*** has a molecular weight of about 28.7 and comprises amino acid 26 through amino acid 286 of the sequence shown in FIG. 6B, SEQ ID NO: 8. The mature OMP-1E protein of E. ***chaffeensis*** has a molecular weight of about 27.8 kDa and comprises amino acid 26 through amino acid 278 of the sequence shown in FIG. 7B, SEQ ID NO: 10. The mature OMP-1F protein of E. ***chaffeensis*** has a molecular weight of about 27.9 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 8B, SEQ ID NO: 12. The mature OMP-1A protein of E. ***chaffeensis*** has a molecular weight of about 29.6 kDa and comprises amino acid 31 through amino acid 297 of the sequence shown in FIG. 9B, SEQ ID NO: 14. The mature OMP-1R protein of E. ***chaffeensis*** has a molecular weight of about 19.7 kDa and comprises amino acid 29 through amino acid 196 of the sequence shown in FIG. 10B, SEQ ID NO: 16. The mature OMP-1S protein of E. ***chaffeensis*** has a molecular weight of about 29.2 kDa and comprises amino acid 26 through amino acid 291 of the sequence shown in FIG. 11B, SEQ ID NO: 18. The OMP-1T protein of E. ***chaffeensis*** comprises the amino acid sequence shown in FIG. 12B, SEQ ID NO: 20. The mature OMP-1U protein of E. ***chaffeensis*** has a molecular weight of about 30.6 kDa and comprises amino acid 26 through amino acid 295 of the sequence shown in FIG. 13B, SEQ ID NO: 22. The mature OMP-1V protein of E. ***chaffeensis*** has a molecular weight of about 28.0 kD and comprises amino acid 27 through amino acid 279 shown in FIG. 14B, SEQ ID NO: 24. The mature OMP-1W protein of E. ***chaffeensis*** has a molecular weight of about 28.8 kDa and comprises amino acid 30 through amino acid 283 of the sequence shown in FIG. 15B, SEQ ID NO: 26. The mature OMP-1X protein of E. ***chaffeensis*** has a molecular weight of about 27.8 kDa and comprises amino acid 25 through amino acid 275 of the sequence shown in FIG. 16B, SEQ ID NO: 28. The mature OMP-1Y protein of E. ***chaffeensis*** has a molecular weight about 28.8 kDa and comprises amino acid 28 through amino acid 285 of the sequence shown in FIG. 17B, SEQ ID NO: 30. The mature OMP-1Z protein of E. ***chaffeensis*** has a molecular weight of about 30.2 kDa and comprises amino acid 27 through amino acid 300 of the sequence. . .

SUMM [0008] The outer membrane proteins from E. ***chaffeensis*** , particularly a recombinant form of OMP-1, are immunogenic and, thus are useful for preparing antibodies. Such antibodies are useful for immunolabeling isolates of E. ***chaffeensis*** and for detecting the presence of E. ***chaffeensis*** in body fluids, tissues, and particularly in monocytes and macrophages. The OMP proteins, particularly OMP-1, are also useful for detecting antibodies to E. ***chaffeensis*** in the blood of patients with clinical signs of

ehrlichiosis . The OMP protein, particularly OMP-1, are also useful immunogens for raising antibodies that are capable of reducing the level of infection in an immunized mammal that has been infected with E. ***chaffeensis*** . The proteins are also useful in a vaccine for protecting against infection with E. ***chaffeensis*** .

SUMM [0009] The ***P30F*** proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. The mature ***P30*** protein of E. ***canis*** has a molecular weight of about 28.8 kDa and comprises amino acid 26 through amino acid 288 of the sequence shown in FIG. 19B, SEQ ID NO: 32. The mature ***P30a*** protein of E. ***canis*** has a molecular weight of about 29.0 kDa and comprises amino acid 26 through amino acid 287 of the sequence shown in FIG. 20B, SEQ ID NO: 34. The mature ***P30*** -1 protein of E. ***canis*** has a molecular weight of about 27.7 kDa and comprises amino acid 55 through amino acid 307 of the sequence shown in FIG. 21B, SEQ ID NO: 36. The mature ***P30*** -2 protein of E. ***canis*** has a molecular weight of about 28.0 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 22B, SEQ ID NO: 38. The mature ***P30*** -3 protein of E. ***canis*** has a molecular weight of about 28.7 kDa and comprises amino acid 26 through amino acid 283 of the sequence shown in FIG. 23B, SEQ ID NO: 40. The mature ***P30*** -4 protein of E. ***canis*** has a molecular weight of about 28.0 kDa and comprises amino acid 26 through amino acid 276 of the sequence shown in FIG. 24B, SEQ ID NO: 42. The mature ***P30*** -5 protein of E. ***canis*** has a molecular weight of about 29.4 kDa and comprises amino acid 27 through amino acid 293 of the sequence shown in FIG. 25B, SEQ ID NO: 44. The mature ***P30*** -6 protein of E. ***canis*** has a molecular weight of about 29.4 kDa and comprises amino acid 31 through amino acid 293 of the sequence shown in FIG. 26B, SEQ ID NO: 54. The mature ***P30*** -7 protein of E. ***canis*** has a molecular weight of about 29.9 kDa and comprises amino acid 31 through amino acid 296 of the sequence shown in FIG. 27B, SEQ ID NO: 56. The mature ***P30*** -8 protein of E. ***canis*** has a molecular weight of about 30.3 kDa and comprises amino acid 27 through amino acid 299 of the sequence shown in FIG. 28B, SEQ ID NO: 46. The mature ***P30*** -9 protein of E. ***canis*** has a molecular weight of about 28.6 kDa and comprises amino acid 27 through amino acid 281 of the sequence shown in FIG. 29B, SEQ ID NO: 58. The mature ***P30*** -10 protein of E. ***canis*** has a molecular weight of about 28.1 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 30B, SEQ ID NO: 48. The mature ***P30*** -11 protein of E. ***canis*** has a molecular weight of about 28.6 kDa and comprises the amino acid 26

through amino acid 279 of sequence shown in FIG. 31B, SEQ ID NO: 60. The ***P30*** -12 protein of E. ***canis*** has a molecular weight of at least 27.3 kDa and comprises the amino acid sequence shown in FIG. 32B, SEQ. . .

SUMM [0010] The ***P30F*** proteins, particularly ***P30*** , are immunogenic and are, thus, useful for preparing antibodies that are useful for immunolabeling isolates of E. ***canis*** . The ***P30*** protein is also useful for diagnosing canine ***ehrlichiosis*** in mammals, particularly in members of the family Canidae, most particularly in dogs and for diagnosing infections with E. ***chaffeensis*** in humans. The ***P30F*** proteins are also useful immunogens for raising antibodies that reduce the level of infection in an immunized mammal that has been infected with E. ***canis*** . The ***P30F*** protein are also useful in a vaccine for protecting animals against infection with E. ***canis*** .

SUMM [0011] The present invention also provides isolated polynucleotides that encode the E. ***chaffeensis*** OMP proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** proteins. The present invention also relates to antibodies which are immunospecific for and bind to the OMP proteins and the ***P30F*** proteins. Such antibodies are useful for immunolabeling isolates of E. ***chaffeensis*** and E. ***canis*** . The present invention also relates to kits containing reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** and to immunogenic compositions containing one or more OMP proteins or ***P30F*** proteins.

DRWD [0012] FIG. 1. shows the DNA sequence and the amino acid sequence encoded by the E. ***chaffeensis*** (p28) gene cloned in pCRIIp28. The N-terminal amino acid sequence of native OMP-1 protein (P28) determined chemically is underlined. Five. . .

DRWD [0013] FIG. 2. shows the restriction map of 6.3-kb genomic DNA including the omp-1 gene copies in E. ***chaffeensis*** . The four DNA fragments were cloned from the genomic DNA (pPS2.6, pPS3.6, pEC2.6, and pEC3.6). A recombinant plasmid pPS2.6 has. . .

DRWD [0030] FIG. 19B shows one embodiment of the ***P30*** protein, FIG. 19A shows one embodiment of the ***P30*** polynucleotide.

DRWD [0031] FIG. 20B shows one embodiment of the ***P30a*** protein, FIG. 20A shows one embodiment of the ***p30a*** polynucleotide.

DRWD [0032] FIG. 21B shows one embodiment of the ***P30*** -1 protein, FIG. 21A shows one embodiment of the ***p30*** -1 polynucleotide.

DRWD [0033] FIG. 22B shows one embodiment of the ***P30*** -2 protein, FIG. 22A shows one embodiment of the ***p30*** -2 polynucleotide.

DRWD [0034] FIG. 23B shows one embodiment of the ***P30*** -3 protein, FIG. 23A shows one embodiment of the ***p30*** -3 polynucleotide.

DRWD [0035] FIG. 24B shows one embodiment of the ***P30*** -4 protein,

FIG. 22A shows one embodiment of the ***p30*** -4 polynucleotide.

DRWD [0036] FIG. 25B shows one embodiment of the ***P30*** -5 protein, FIG. 22A shows one embodiment of the ***p30*** -5 polynucleotide.

DRWD [0037] FIG. 26B shows one embodiment of the ***P30*** -6 protein, FIG. 26A shows one embodiment of the ***p30*** -6 polynucleotide.

DRWD [0038] FIG. 27B shows one embodiment of the ***P30*** -7 protein, FIG. 27A shows one embodiment of the ***p30*** -7 polynucleotide.

DRWD [0039] FIG. 28B shows one embodiment of the ***P30*** -8 protein, FIG. 28A shows one embodiment of the ***p30*** -8 polynucleotide.

DRWD [0040] FIG. 29B shows one embodiment of a portion of the ***P30*** -9 protein, FIG. 29A shows one embodiment of the ***p30*** -9 polynucleotide.

DRWD [0041] FIG. 30B shows one embodiment of a portion of the ***P30*** -10 protein, FIG. 30A shows one embodiment of the ***p30*** -10 polynucleotide encoding such protein.

DRWD [0042] FIG. 31B shows one embodiment of a portion of the ***P30*** -11 protein, FIG. 31A shows one embodiment of the ***p30*** -11 polynucleotide.

DRWD [0043] FIG. 32B shows one embodiment of a portion of the ***P30*** -12 protein, FIG. 32A shows one embodiment of the ***p30*** -12 polynucleotide.

DRWD [0045] FIG. 34 depicts the amino acid sequences alignment of six E. ***chaffeensis*** OMP-1s and Cowdria ruminantium MAP-1. Aligned positions of identical amino acids with OMP-1F are shown with dots. The sequence of. . .

DETD [0046] The present invention provides a group of outer membrane proteins of E. ***chaffeensis*** , OMP proteins, and a group of outer membrane proteins of E. ***canis*** , the ***P30F*** proteins. The mature OMP-1 protein of E. ***chaffeensis*** has a molecular weight of about 27.7 kDa and comprises amino acid 26 through amino acid 281 of the sequence shown in FIG. 3B, SEQ ID NO: 2. The mature OMP-1B protein of E. ***chaffeensis*** has a molecular weight of about 28.2 kDa and comprises amino acid 26 through amino acid 283 of the sequence shown in FIG. 4B, SEQ ID NO: 4. The mature OMP-1C protein of E. ***chaffeensis*** has a molecular weight of about 27.6 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 5B, SEQ ID NO: 6. The mature OMP-1D protein of E. ***chaffeensis*** has a molecular weight of about 28.7 and comprises amino acid 26 through amino acid 286 of the sequence shown in FIG. 6B, SEQ ID NO: 8. The mature OMP-1E protein of E. ***chaffeensis*** has a molecular weight of about 27.8 kDa and comprises amino acid 26 through amino acid 278 of the sequence shown in FIG. 7B, SEQ ID NO: 10. The mature OMP-1F protein of E. ***chaffeensis*** has a molecular weight of about 27.9 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 8B, SEQ ID NO: 12. The mature OMP-1A protein

of E. ***chaffeensis*** has a molecular weight of about 29.6 kDa and comprises amino acid 31 through amino acid 279 of the sequence shown in FIG. 9B, SEQ ID NO: 14. The mature OMP-1R protein of E. ***chaffeensis*** has a molecular weight of about 19.7 kDa and comprises the amino acid 29 through amino acid 196 of the sequence shown in FIG. 10B, SEQ ID NO: 16. The mature OMP-1S protein of E. ***chaffeensis*** has a molecular weight of about 29.2 kDa and comprises amino acid 26 through amino acid 291 of the sequence shown in FIG. 11B, SEQ ID NO: 18. The OMP-1T protein of E. ***chaffeensis*** comprises the amino acid sequence shown in FIG. 12B, SEQ ID NO: 20. The mature OMP-1U protein of E. ***chaffeensis*** has a molecular weight of about 30.6 kDa and comprises amino acid 26 through amino acid 295 of the sequence shown in FIG. 13B, SEQ ID NO: 22. The mature OMP-1V protein of E. ***chaffeensis*** has a molecular weight of about 28.0 kD and comprises amino acid 27 through amino acid 279 shown in FIG. 14B, SEQ ID NO: 24. The mature OMP-1W protein of E. ***chaffeensis*** has a molecular weight of about 28.8 kDa and comprises amino acid 30 through amino acid 283 of the sequence shown in FIG. 15B, SEQ ID NO: 26. The mature OMP-1X protein of E. ***chaffeensis*** has a molecular weight of about 27.8 kDa and comprises amino acid 25 through amino acid 275 of the sequence shown in FIG. 16B, SEQ ID NO: 28. The mature OMP-1Y protein of E. ***chaffeensis*** has a molecular weight about 28.8 kDa and comprises amino acid 28 through amino acid 285 of the sequence shown in FIG. 17B, SEQ ID NO: 30. The mature OMP-1Z protein of E. ***chaffeensis*** has a molecular weight of about 30.2 kDa and comprises amino acid 27 through amino acid 300 of the sequence. . .

DETD [0047] The mature ***P30*** protein of E. ***canis*** has a molecular weight of about 28.8 kDa and comprises amino acid 26 through amino acid 288 of the sequence shown in FIG. 19B, SEQ ID NO: 32. The mature ***P30a*** protein of E. ***canis*** has a molecular weight of about 29.0 kDa and comprises amino acid 26 through amino acid 287 of the sequence shown in FIG. 20B, SEQ ID NO: 34. The mature ***P30*** -1 protein of E. ***canis*** has a molecular weight of about 27.7 kDa and comprises amino acid 55 through amino acid 307 of the sequence shown in FIG. 21B, SEQ ID NO: 36. The mature ***P30*** -2 protein of E. ***canis*** has a molecular weight of about 28.0 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 22B, SEQ ID NO: 38. The mature ***P30*** -3 protein of E. ***canis*** has a molecular weight of about 28.7 kDa and comprises amino acid 26 through amino acid 283 of the sequence shown in FIG. 23B, SEQ ID NO: 40. The mature ***P30*** -4 protein of E. ***canis*** has a molecular weight of about 28.0 kDa and comprises amino acid 26 through amino acid 276 of the sequence shown in FIG. 24B, SEQ ID NO: 42. The mature ***P30*** -5 protein of E. ***canis*** has a molecular weight of about 29.4 kDa and comprises amino acid 27 through amino acid

293 of the sequence shown in FIG. 25B, SEQ ID NO: 44. The mature ***P30*** -6 protein of E. ***canis*** has a molecular weight of about 29.4 kDa and comprises amino acid 31 through amino acid 293 of the sequence shown in FIG. 26B, SEQ ID NO: 54. The mature ***P30*** -7 protein of E. ***canis*** has a molecular weight of about 29.9 kDa and comprises amino acid 31 through amino acid 296 of the sequence shown in FIG. 27B, SEQ ID NO: 56. The mature ***P30*** -8 protein of E. ***canis*** has a molecular weight of about 30.3 kDa and comprises amino acid 27 through amino acid 299 of the sequence shown in FIG. 28B, SEQ ID NO: 46. The mature ***P30*** -9 protein of E. ***canis*** has a molecular weight of about 28.6 kDa and comprises amino acid 27 through amino acid 281 of the sequence shown in FIG. 29B, SEQ ID NO: 58. The mature ***P30*** -10 protein of E. ***canis*** has a molecular weight of about 28.1 kDa and comprises amino acid 26 through amino acid 280 of the sequence shown in FIG. 30B, SEQ ID NO: 48. The mature ***P30*** -11 protein of E. ***canis*** has a molecular weight of about 28.6 kDa and comprises the amino acid 26 through amino acid 279 of sequence shown in FIG. 31B, SEQ ID NO: 60. The ***P30*** -12 protein of E. ***canis*** has a molecular weight of at least 27.3 kDa and comprises the amino acid sequence shown in FIG. 32B, SEQ. . .

DETD . . . The present invention also encompasses variants of the OMP proteins shown in FIGS. 3-18 and 33 and variants of the ***P30F*** proteins shown in FIGS. 19-32. A "variant" as used herein, refers to a protein whose amino acid sequence is similar. . .

DETD . . . amino acids are added to the amino or carboxy terminus of the amino acid sequence of an OMP protein, a ***P30F*** protein, or a variant of such protein. Typically, such additions are made to stabilize the resulting fusion protein or to simplify purification of an expressed recombinant form of the corresponding OMP protein, ***P30F*** protein or variant of such protein. Such tags are known in the art. Representative examples of such tags include sequences. . .

DETD [0052] The present invention also encompasses OMP proteins and ***P30F*** proteins in which one or more amino acids, preferably no more than 10 amino acids, in the respective OMP protein or ***P30F*** are altered by posttranslation processes or synthetic methods. Examples of such modifications include, but are not limited to, acetylation, amidation,. . .

DETD . . . of OMP-1, are immunogenic and, thus are useful for preparing antibodies. Such antibodies are useful for immunolabeling isolates of E. ***chaffeensis*** and for detecting the presence of E. ***chaffeensis*** in body fluids, tissues, and particularly in monocytes and macrophages. The OMP proteins, particularly OMP-1, are also useful for detecting antibodies to E. ***chaffeensis*** in the blood of patients with clinical signs of ***ehrlichiosis*** . The OMP

proteins , particularly OMP-1, are also useful immunogens for raising antibodies that are capable of reducing the level of infection in an immunized mammal that has been infected with E. ***chaffeensis*** . The OMP proteins are also useful in a vaccine for protecting against infection with E. ***chaffeensis*** .

DETD [0054] The ***P30F*** proteins, particularly recombinant forms of ***P30*** , are immunogenic and are, thus, useful for preparing antibodies that are useful for immunolabeling isolates of E. ***canis*** . The ***P30*** protein is also useful for diagnosing canine ***ehrlichiosis*** in mammals, particularly in members of the family Canidae, most particularly in dogs and for diagnosing infections with E. ***chaffeensis*** in humans. The ***P30F*** proteins are also useful immunogens for raising antibodies that reduce the level of infection in an immunized mammal that has been infected with E. ***canis*** . The ***P30F*** proteins are also useful in a vaccine for protecting animals against infection with E. ***canis*** .

DETD . . . amino acid 251 of the OMP-1 protein and which derivative binds to antibodies in sera from humans infected with E. ***chaffeensis***

DETD [0057] The present invention also provides isolated polynucleotides which encode the OMP proteins and the ***P30F*** proteins. The OMP-1 polynucleotide encodes the OMP-1 protein of E. ***chaffeensis*** , FIG. 3A shows one embodiment of the OMP-1 polynucleotide, SEQ ID NO: 1. The OMP-1B polynucleotide encodes the OMP-1B protein of E. ***chaffeensis*** ; FIG. 4A shows one embodiment of the OMP-1B polynucleotide, SEQ ID NO: 3. The OMP-1C polynucleotide encodes the OMP-1C protein of E. ***chaffeensis*** , FIG. 5A shows one embodiment of the OMP-1C polynucleotide; SEQ ID NO: 5. The OMP-1D polynucleotide encodes the OMP-1D protein of E. ***chaffeensis*** ; FIG. 6A shows one embodiment of the OMP-1D polynucleotide, SEQ ID NO: 7. The OMP-1E polynucleotide encodes the OMP-1E protein of E. ***chaffeensis*** ; FIG. 7A shows one embodiment of the OMP-1E polynucleotide, SEQ ID NO: 9. The OMP-1F polynucleotide encodes the OMP-1F protein of E. ***chaffeensis*** ; FIG. 8A shows one embodiment of the OMP-1F polynucleotide, SEQ ID NO: 11. The OMP-1A polynucleotide encodes the OMP-1A protein of E. ***chaffeensis*** ; FIG. 9A shows one embodiment of the OMP-1A polynucleotide, SEQ ID NO: 13. The OMP-1R polynucleotide encodes the OMP-1R. . . of a portion of the OMP-1R polynucleotide, SEQ ID NO: 15. The OMP-1S polynucleotide encodes the OMP-1S protein of E. ***chaffeensis*** ; FIG. 11A shows one embodiment of a portion of the OMP-1S polynucleotide, SEQ ID NO: 17. The OMP-1T polynucleotide encodes the OMP-1T protein of E. ***chaffeensis*** ; FIG. 12A shows one embodiment of a portion of the OMP-1T polynucleotide, SEQ ID NO: 19. The OMP-1U polynucleotide encodes the OMP-1U protein of E. ***chaffeensis*** ; FIG. 13A shows one

embodiment of the OMP-1U polynucleotide, SEQ ID NO: 21. The OMP-1V polynucleotide encodes the OMP-1V protein of E. ***chaffeensis*** ; FIG. 14A shows one embodiment of the OMP-1V polynucleotide, SEQ ID NO: 23. The OMP-1W polynucleotide encodes the OMP-1W protein of E. ***chaffeensis*** ; FIG. 15A shows one embodiment of the OMP-1W polynucleotide, SEQ ID NO: 25. The OMP-1X polynucleotide encodes an OMP-1X protein of E. ***chaffeensis*** ; FIG. 16A shows one embodiment of the OMP-1X polynucleotide, SEQ ID NO 27. The OMP-1Y polynucleotide encodes the OMP-1Y protein of E. ***chaffeensis*** ; FIG. 17A shows one embodiment of the OMP-1Y polynucleotide, SEQ ID NO 29. The OMP-1Z polynucleotide encodes the OMP-1Z protein of E. ***chaffeensis*** ; FIG. 18A shows one embodiment of an OMP-1Z polynucleotide encoding such polypeptide, SEQ ID NO: 49. The OMP-1H polynucleotide encodes the OMP-1H protein of E. ***chaffeensis*** ; FIG. 33A shows one embodiment of a portion of the OMP-1H polynucleotide, SEQ ID NO: 51.

DETD [0058] The ***p30*** polynucleotide encodes the ***P30*** protein of E. ***canis*** , FIG. 19A shows one embodiment of the ***p30*** polynucleotide, SEQ ID NO: 31. The ***p30a*** polynucleotide encodes the ***P30a*** protein of E. ***canis*** , FIG. 20A shows one embodiment of the ***p30a*** polynucleotide, SEQ ID NO: 33. The ***p30*** -1 polynucleotide encodes the ***P30*** -1 protein of E. ***canis*** ; FIG. 21A shows one embodiment of the ***p30*** -1 polynucleotide, SEQ ID NO: 35. The ***p30*** -2 polynucleotide encodes the ***P30*** -2 protein of E. ***canis*** ; FIG. 22A shows one embodiment of the ***p30*** -2 polynucleotide, SEQ ID NO: 37. The ***p30*** -3 polynucleotide encodes the ***P30*** -3 protein of E. ***canis*** ; FIG. 23A shows one embodiment of the ***p30*** -3 polynucleotide, SEQ ID NO: 39. The ***p30*** -4 polynucleotide encodes the ***P30*** -4 protein of E. ***canis*** , FIG. 24A shows one embodiment of the ***p30*** -4 polynucleotide, SEQ ID NO: 41. The ***p30*** -5 polynucleotide encodes the ***P30*** -5 protein of E. ***canis*** , FIG. 25A shows one embodiment of the ***p30*** -5 polynucleotide, SEQ ID NO: 43. The ***p30*** -6 polynucleotide encodes the ***P30*** -6 protein, FIG. 26A shows one embodiment of the ***p30*** -6 polynucleotide, SEQ ID NO: 53. The ***p30*** -7 polynucleotide encodes the ***P30*** -7 protein of E. ***canis*** ; FIG. 27A shows one embodiment of the ***p30*** -7 polynucleotide, SEQ ID NO: 55. The ***p30*** -8 polynucleotide encodes the ***P30*** -8 protein of E. ***canis*** ; FIG. 28A shows one embodiment of the ***p30*** -8 polynucleotide, SEQ ID NO: 45. The ***p30*** -9 polynucleotide encodes the ***P30*** -9 protein of E. ***canis*** ; FIG. 29A shows one embodiment of a portion of the ***p30*** -9 polynucleotide, SEQ ID NO: 57. The ***p30*** -10 polynucleotide

encodes the ***P30*** -10 protein of E. ***canis*** , FIG. 30A shows one embodiment of a portion of the ***p30*** -10 polynucleotide, SEQ ID NO: 47. The ***p30*** -11 polynucleotide encodes the ***P30*** -11 protein of E. ***canis*** ; FIG. 31A shows one embodiment of a portion of the ***p30*** -11 polynucleotide, SEQ ID NO: 59. The ***p30*** -12 polynucleotide encodes the ***P30*** -12 protein of E. ***canis*** ; FIG. 32A shows one embodiment of a portion of the ***p30*** -12 polynucleotide, SEQ ID NO: 61.

DETD [0059] The polynucleotides are useful for producing the outer membrane proteins of E. ***chaffeensis*** and E. ***canis*** . For example, an RNA molecule encoding the outer membrane protein OMP-1 is used in a cell-free translation systems to prepare. . .

DETD [0061] Polynucleotides encoding the OMP proteins and the ***P30F*** proteins are also useful for designing hybridization probes for isolating and identifying cDNA clones and genomic clones encoding the OMP proteins, the ***P30F*** proteins or allelic forms thereof. Such hybridization techniques are known to those of skill in the art. The sequences that encode the OMP proteins and the ***P30F*** proteins are also useful for designing primers for polymerase chain reaction (PCR), a technique useful for obtaining large quantities of cDNA molecules that encode the OMP proteins and the ***P30F*** proteins.

DETD . . . antisense polynucleotides, having sequences which are complementary to the DNA and RNA sequences which encode the OMP proteins and the ***P30F*** proteins. The term complementary as used herein refers to the natural binding of the polynucleotides under permissive salt and temperature. . .

DETD . . . as primers in polymerase chain reaction (PCR) technologies to amplify transcripts of the genes which encode the OMP proteins, the ***P30F*** proteins or portions of such transcripts. Preferably, the primers comprise 18-30 nucleotides, more preferably 19-25 nucleotides. Preferably, the primers have. . . 98% complementary with a portion of the DNA strand, i.e., the sense strand, which encodes the OMP protein or the ***P30F*** protein, or a portion of its corresponding antisense strand. Preferably, the primer has at least 99% complementarity, more preferably 100%. . . which have 100% complementarity with the antisense strand of a double-stranded DNA molecule which encodes an OMP protein or a ***P30F*** protein have a sequence which is identical to a sequence contained within the sense strand. The identity of primers which. . .

DETD . . . encompasses oligonucleotides that are useful as hybridization probes for detecting transcripts of the genes which encode the OMP proteins and ***P30F*** proteins or for mapping of the genes which encode the OMP proteins and ***P30F*** proteins. Preferably, such oligonucleotides comprise at least 210 nucleotides, more preferably at

least 230, most preferably from about 210 to. . . complementary with a sequence contained within the sense strand of a DNA molecule which encodes each of OMP proteins and ***P30F*** proteins or with a sequence contained within its corresponding antisense strand. Such hybridization probes bind to the sense strand under. . . 20.degree. C. to 25.degree. C. below Tm. The probes are used in Northern assays to detect transcripts of OMP and ***P30F*** homologous genes and in Southern assays to detect OMP and ***P30F*** homologous genes. The identity of probes which are 200 nucleotides in length and have full complementarity with a portion of. . .

DETD . . . The present invention also encompasses isolated polynucleotides which are alleles of the genes which encode the OMP proteins and the ***P30F*** proteins. As used herein, an allele or allelic sequence is an alternative form of the gene which may result from one or more mutations in the sequences which encode the OMP proteins and ***P30F*** proteins. Such mutations typically arise from natural addition, deletion of substitution of nucleotides in the open reading frame sequences. Any. . . such as for example screening libraries with probes having sequences identical to or complementary with one or more OMP or ***P30F*** polynucleotides.

DETD [0066] The present invention also encompasses altered polynucleotides which encode OMP proteins and ***P30F*** proteins. Such alterations include deletions, additions, or substitutions. Such alterations may produce a silent change and result in an OMP protein or ***P30F*** protein having the same amino acid sequence as the OMP protein or ***P30F*** protein encoded by the unaltered polynucleotide. Such alterations may produce a nucleotide sequence possessing non-naturally occurring codons. For example, codons. . . may also introduce new restriction sites into the sequence or result in the production of an OMP protein variant or ***P30F*** protein variant. Typically, such alterations are accomplished using site-directed mutagenesis.

DETD . . . aspect, the present invention relates to antibodies which are specific for and bind to at least one OMP protein or ***P30F*** protein. Such antibodies are useful research tools for identifying cells, particularly monocytes or macrophages, infected with E. ***chaffeensis*** or E. ***canis*** and for purifying the major outer membrane protein of E. ***chaffeensis*** or E. ***canis*** from partially purified preparations by affinity chromatography. Such antibodies are also useful for identifying bacterial colonies, particularly colonies of genetically-engineered bacteria, that are expressing the major outer membrane protein of E. ***chaffeensis*** or E. ***canis*** .

DETD [0070] The present invention also relates to kits containing reagents for diagnosing E. ***chaffeensis*** and E. ***canis*** . The kit comprises one or more OMP proteins, or one or more E. ***canis***

proteins, or antigenic fragments thereof. For ease of detection, it is preferred that the OMP protein or ***P30F*** proteins be attached to a substrate such as a column, plastic dish, matrix, or membrane, preferably nitrocellulose. The kit may further comprise a biomolecule, preferably a secondary antibody, for detecting interactions between the isolated OMP protein or ***P30F*** protein and antibodies in a patient sample. Preferably, the biomolecule is coupled to a detectable tag such as an enzyme, chromophore, fluorophore, or radio-isotope. The kit is used by contacting a patient sample with the OMP protein or ***P30F*** protein under conditions that permit formation of antigen-antibody complexes. Then the biomolecule is added and the presence or absence of. . .

DETD [0072] The present invention also provides a method for detecting antibodies to the E. ***chaffeensis*** or E. ***canis*** in a sample of a bodily fluid from a patient. The method comprises providing an isolated outer membrane protein of E. ***chaffeensis*** or E. ***canis*** , particularly a recombinant form of the isolated protein, contacting the outer membrane protein or polypeptide with a sample taken from. . . such as for example, an enzyme, fluorophore, or chromophore. Formation of the complex is indicative of the presence of anti-E ***chaffeensis*** or anti-E ***canis*** antibodies, either IgM or IgG, in the patient. Thus, the method is used to determine whether a patient is infected with E. ***chaffeensis*** or E. ***canis*** .

DETD . . . high quality antigen. Accordingly, it is more advantageous to use a recombinant form of the outer membrane protein of E. ***chaffeensis*** or E. ***canis*** since such proteins, typically, are more pure and consistent in quality than a purified form of such protein.

DETD [0075] The present invention also relates to immunogenic compositions comprising one or more OMP protein of E. ***chaffeensis*** and a pharmaceutically acceptable adjuvant and to immunogenic compositions comprising one or more ***P30F*** proteins of E. ***canis*** and a pharmaceutically acceptable adjuvant, which, preferably, enhances the immunogenic activity of the outer membrane protein in the host animal.

DETD [0076] Preparing the OMP Proteins and the ***P30F*** Proteins

DETD [0077] The OMP proteins and ***P30F*** proteins may be produced by conventional peptide synthesizers. The OMP proteins and ***P30F*** proteins may also be produced using cell-free translation systems and RNA molecules derived from DNA constructs that encode the OMP proteins and ***P30F*** proteins. Alternatively, OMP proteins and ***P30F*** proteins are made by transfecting host cells with expression vectors that comprise a DNA sequence that encodes the respective OMP protein or ***P30F*** protein and then inducing expression of the protein in the host cells. For recombinant production,

recombinant constructs comprising one or more of the sequences which encode the OMP protein or ***P30F*** protein are introduced into host cells by conventional methods such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic. . .

DETD [0078] The OMP proteins or ***P30F*** proteins may be expressed in suitable host cells, such as for example, mammalian cells, yeast, bacteria, or other cells under. . . disrupted by physical or chemical means, and the resulting crude extract retained for further purification of the OMP protein or ***P30F*** protein.

DETD . . . chromatography steps, and high performance liquid chromatography (HPLC), and affinity chromatography may be used to isolate recombinant OMP protein or ***P30F*** protein

DETD [0081] The OMP proteins, ***P30F*** proteins, and variants thereof are used as immunogens to produce antibodies immunospecific for one or more OMP protein or one or more ***P30F*** protein. The term "immunospecific" means the antibodies have substantially greater affinity for one or more OMP protein or ***P30F*** protein than for other proteins. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, and. . .

DETD [0082] Polyclonal antibodies are generated using conventional techniques by administering the OMP protein or ***P30F*** protein, or a chimeric molecule to a host animal. Depending on the host species, various adjuvants may be used to. . .

DETD . . . involve competitive binding or immunoradiometric assays and typically involve the measurement of complex formation between the respective OMP protein or ***P30F*** protein and the antibody.

DETD [0085] Polynucleotides that Encode OMP Proteins and ***P30F*** Proteins

DETD [0086] Polynucleotides comprising sequences encoding an OMP protein or ***P30F*** protein may be synthesized in whole or in part using chemical methods. Polynucleotides which encode an OMP protein or ***P30F*** protein, particularly alleles of the genes which encode an OMP protein or ***P30F*** protein, may be obtained by screening a genomic library of an E. ***chaffeensis*** or E. ***canis*** isolate with a probe comprising sequences identical or complementary to the sequences shown in FIGS. 3-33 or with antibodies immunospecific for a OMP protein or ***P30F*** protein to identify clones containing such polynucleotide.

DETD [0087] Polynucleotides which Encode OMP-1 Protein and ***P30*** Protein

DETD [0089] E. ***chaffeensis*** Arkansas strain and E. ***canis*** Oklahoma strain were cultivated in the DH82 dog macrophage cell line and purified by Percoll density gradient centrifugation. Purified ***ehrlichiae*** (100 .mu.g) were suspended with 10 mM sodium

phosphate buffer, pH 7.4, containing 0.1% Sodium N-lauroyl sarcosine (Sarkosyl) [Sigma, St.. . .

DETD [0090] Transmission electron microscopy revealed that the purified ***ehrlichial*** fraction consists of a mixture of electron dense and light forms of E. ***chaffeensis*** with slight disintegration of inner membrane. ***Ehrlichiae*** were not surrounded with the host inclusion membrane. Various sizes of membrane vesicles (<1 .mu.m) without significant ribosomes or nuclear. . . moles/min/mg of protein in the Percoll-purified organisms, suggesting that the insoluble fraction primarily consisted of the outer membrane of E. ***chaffeensis*** .

DETD [0091] Analysis of the Sarkosyl-soluble, and insoluble fraction of E. ***chaffeensis*** by SDS-PAGE suggested that proteins of 30-kDa range in the insoluble fraction represent the major outer membrane proteins of this organism. Analysis of the Sarkosyl-soluble, and insoluble fraction of E. ***canis*** by SDS-PAGE suggested that proteins of 30-kDa range in the insoluble fraction represent the major outer membrane proteins of this organism also. E. ***canis*** was antigenically cross reactive with E. ***chaffeensis*** . These findings indicate that the 30-kDa range proteins represent the major outer membrane proteins of these two ***Ehrlichia*** spp.

DETD . . . To improve resolution of the outer membrane proteins, proteins in the Sarkosyl-insoluble pellet prepared from 400 .mu.g of purified E. ***chaffeensis*** were separated by a reversed-discontinuous (Rd) SDS-PAGE (2.5-cm-long 17% gel on top of 11-cm-long 12% gel). At least five proteins of 30-kDa range in E. ***chaffeensis*** (P23, P25, P27, P28, and P29) were resolved from the Sarkosyl-insoluble proteins.

DETD [0095] Genomic DNA of E. ***chaffeensis*** was isolated from purified organisms. PCR amplification with FECH1 and RECH2 primers was performed using a Perkin-Elmer Cetus DNA Thermal. . .

DETD [0097] A DNA fragment comprising the partial ***p30*** gene was prepared in a similar manner, i.e., by PCR amplification of genomic DNA of E. ***canis*** using the forward primer, FECH1, which is described above, and a reverse primer, REC1, which is complimentary to the DNA sequence corresponding to amino acid positions 185 to 191 of the mature OMP-1 of E. ***chaffeensis*** . The sequence of REC1 is 5'-ACCTAACTTTCCTTGGTAAG-3', SEQ ID NO: 66.

DETD [0098] Genomic DNA of E. ***canis*** was isolated from the purified organism. PCR amplification was performed by using a Perkin-Elmer Cetus DNA Thermal Cycler (model 480).. . .

DETD [0099] The 0.6-kb DNA fragment containing a partial ***p30*** gene cloned had an open reading frame (ORF) of 579 bp encoding a 193-amino-acid protein with a molecular mass of 21,175 Da. The partial ***P30*** protein of E. ***canis*** was encoded by nucleotide 97 through nucleotide 672 of the sequence shown in FIG. 19A and comprised

amino acid 33. . .

DETD [0102] Genomic DNA extracted from the purified E. ***chaffeensis*** (200 ng each) was digested with restriction endonucleases, electrophoresed, and transferred to Hybond-N.sup.+ nylon membrane (Amersham, Arlington Heights, Ill.), by. . .

DETD [0103] Genomic Southern blot analysis with several restriction enzymes resulted in one or more DNA fragment(s) of E. ***chaffeensis*** which hybridized to .sup.32P-labeled omp-1 gene probe. The restriction enzymes used did not cut within the p28 gene portion of. . .

DETD [0104] B. Cloning and sequencing of genomic copies of E. ***chaffeensis*** omp-1 gene.

DETD . . . positive clones were isolated from the transformnant. The positive clones were designated pEC2.6; pEC3.6, pPS2.6, and pPS3.6. These contained the ***ehrlichial*** DNA fragments of 2.6-kb (EcoR I), 3.6 kb (EcoR I), 2.6 kb (Pst I), and 3.6 kb (Pst I), respectively.. . . clones pEC3.6 and pPS2.6 overlapped as shown in FIG. 2. The overlapping area was further confirmed by PCR of E. ***chaffeensis*** genomic DNA with two pairs of primer sets interposing the junctions of the four clones. The 1.1- to 1.6-kb DNA. . .

DETD . . . Four DNA fragments from 2.6 to 3.6 kb were cloned from the EcoRI-digested and the PstI-digested genomic DNA of E. ***chaffeensis*** by colony hybridization with radiolabeled omp-1 gene probe. The inserted DNA of the two recombinant clones, pEC3.6 and PPS2.6, were. . . (designated omp-1B to omp-1F), which are tandemly-arrayed and are homologous to the p28 gene (but are not identical), in the ***ehrlichial*** genomic DNA of 6,292 bp. The intergenic spaces were 581 bp between omp-1A and omp-1B and 260-308 bp among others.. . .

DETD . . . sequence in omp-1F gene (the 80th to 94th amino acids) was identical to the N-terminal amino acid sequences of E. ***chaffeensis*** native P23 protein as determined chemically, which indicates that P23 is derived from the omp-1F gene.

DETD [0111] Alignment of predicted amino acid sequences of the E. ***chaffeensis*** OMP-1 family and Cowdria ruminantium, revealed substitutions or deletions of one or several contiguous amino acid residues throughout the molecules.. . .

DETD [0113] Preparation of a Recombinant Form of OMP-1 and ***P30***

DETD [0114] The 0.8-kb p28 gene from E. ***chaffeensis*** was excised from the clone pCRIIp28 by EcoRI-NotI double-digestion, ligated into EcoRI-NotI sites of a pET 29a expression vector, and. . .

DETD [0115] An expression vector comprising the ***p30*** gene was used to prepare the recombinant form of ***P30*** . To prepare the expression vector, an 0.6-kb fragment was excised from the clone pCRIIp30 by EcoRI digestion, ligated into EcoRI. . . acid sequence consisting of 249-amino acid residues with a molecular mass of 27,316

Da. The amino acid sequence of the ***P30*** portion of the fusion protein, referred to hereinafter as rP30, is amino acid 33 through amino acid 224 of the. . .

DETD . . . the rabbit anti-rOMP-1 antibody recognized not only rOMP-1 (31 kDa) and OMP-1 protein, but also P29 and P25 of E. ***chaffeensis*** and ***P30*** of E. ***canis*** . These results indicate that OMP-1 shares antigenic epitopes with P25 and P29 in E. ***chaffeensis*** and ***P30*** of E. ***canis*** .

DETD [0121] Convalescent-phase serum from a patient with clinical signs of human ***ehrlichiosis*** was used. Western blot analyses using the rP28 protein as antigen was performed with 1:1,000 dilutions of this serum. Alkaline. . . a 1:1,000 or 1:2,000 dilution as secondary antibodies. Results indicated that serum from a patient with clinical signs of human ***ehrlichiosis*** reacted strongly to rOMP-1 protein (31 kDa).

DETD [0123] Convalescent-phase serum from a patient with clinical signs of human ***ehrlichiosis*** was reacted with the rP30 protein of E. ***canis*** as described in Example 1. The serum reacted strongly to rP30. These results indicate the rP30 is useful for diagnosing an infection with E. ***chaffeensis*** in human patients.

DETD [0124] Identifying E. ***chaffeensis*** -Infected Cells using Anti-rOMP-1 Antibody

DETD [0125] E. ***chaffeensis*** -infected DH82 cells were sonicated and centrifuged at 400.times.g for 10 min. The supernatant was then centrifuged at 10,000.times.g for 10 min to obtain ***ehrlichia*** -enriched pellet. The pellet was resuspended and incubated with rabbit anti-rOMP-1 antibody or normal rabbit serum (1:100 dilution) at 37.degree. C. for 1 h in PBS containing 1% bovine serum albumin (BSA-PBS). After washing, the ***ehrlichiae*** was incubated with gold-conjugated protein G (20 nm), Sigma) at 1:30 dilution for 1 h at room temperature in BSA-PBS.. . .

DETD [0126] Transmission immunoelectron microscopy with colloidal gold-conjugated protein G and rabbit anti-rP28 antibody revealed gold particles bound to E. ***chaffeensis*** surface. The distribution of the particles was random, close to the surface, and appeared as if almost embedded in the. . . and thus, could be recognized by rabbit anti-rOMP-1 antibody. No gold particles were observed on host cytoplasmic membrane or E. ***chaffeensis*** incubated with normal rabbit serum.

DETD [0127] Immunization of Mice and E. ***chaffeensis*** Challenge

DETD . . . adjuvant. Four mice were intraperitoneally injected with a mixture of the minced gel without protein and the respective adjuvants. For ***ehrlichia*** -challenge, approximately 1.times.10.sup.7 DH82 cells heavily-infected with E. ***chaffeensis*** were disrupted by sonication in serum-free DMEM (GIBCO-BRL) and centrifuged at 200.times.g

for 5 min. The supernatant was diluted to. . . each mouse 10 days after the last immunization. Before challenge, all 5-immunized mice had a titer of 1:160 against E. ***chaffeensis*** antigen by IFA and all 4-nonimmunized mice were negative.

DETD . . . of blood was collected in an EDTA tube from each mouse and protection was assessed by PCR detection of E. ***chaffeensis*** 16S rDNA in the buffy coat of the collected blood. E. ***chaffeensis*** could not be reisolated in cell culture at day 10 postinfection. Day 5 post challenge is the optimum time at which establishment of ***ehrlichial*** infection can be examined by PCR without the influence of residual DNA from the ***ehrlichiae*** used as the challenge before the spontaneous clearance of organisms take place. The E. ***chaffeensis*** -specific DNA fragment was observed in all nonimmunized mice but not in any immunized mice, indicating that immunization of rOMP-1 apparently protects mice from ***ehrlichial*** infection and indicating that the OMP-1 is a potential protective antigen.

DETD [0130] Assaying for the Presence of Anti- ***P30*** Antibody in Dogs

DETD . . . as an antigen in a Western immunoblot analysis and dot blot analysis to detect the presence of antibody to E. ***canis*** in serum from E. ***canis*** infected dogs. The results of the Western immunoblot analysis indicated that reactivity of the sera with rP30 was stronger than the reactivity that was observed when purified E. ***canis*** was used as antigen. The results of the dot blot assay indicated that rP30 is a useful and sensitive tool for serodiagnosis of canine ***ehrlichiosis*** .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 1

LENGTH: 846

TYPE: DNA

ORGANISM: ***Ehrlichia*** ***chaffeensis***

FEATURE:

NAME/KEY: CDS

LOCATION: (1)..(846)

SEQUENCE: 1

atg aat tac aaa aaa gtt ttc ata aca agt gca ttg ata tca tta ata 48. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 2

LENGTH: 281

TYPE: PRT

ORGANISM: ***Ehrlichia*** ***chaffeensis***

SEQUENCE: 2

Met Asn Tyr Lys Lys Val Phe Ile Thr Ser Ala Leu Ile Ser Leu Ile

1 5. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 3
LENGTH: 852
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(852)
SEQUENCE: 3
atg aat tac aag aaa att ttt gta agc agt gca tta att tca tta atg 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 4
Met Asn Tyr Lys Lys Ile Phe Val Ser Ser Ala Leu Ile Ser Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 843
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(843)
SEQUENCE: 5
atg aac tgc aaa aaa ttt ttt ata aca act gca ttg gca ttg cca atg 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 6
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Ala Leu Pro Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7
LENGTH: 861
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(861)
SEQUENCE: 7
atg aac tgc gaa aaa ttt ttt ata aca act gca tta aca tta cta atg 48. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 8
LENGTH: 286
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 8
Met Asn Cys Glu Lys Phe Phe Ile Thr Thr Ala Leu Thr Leu Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 9
LENGTH: 837
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(837)
SEQUENCE: 9
atg aat tgc aaa aaa ttt ttt ata aca act gca tta gta tca cta atg 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 10
LENGTH: 278
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 10
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Val Ser Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 11
LENGTH: 843
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(843)
SEQUENCE: 11
atg aat tgc aaa aaa ttt ttt ata aca act aca tta gta tcg cta atg 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 12
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 12
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Thr Leu Val Ser Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 13
LENGTH: 894
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(894)
SEQUENCE: 13
atg gaa aat ctc atg aat aag aaa aac aaa ttc ttt aca ata agt aca 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 14
LENGTH: 297
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 14
Met Glu Asn Leu Met Asn Lys Lys Asn Lys Phe Phe Thr Ile Ser Thr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 15
LENGTH: 591
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(591)
SEQUENCE: 15
atg ata tat aaa gaa aaa ctt act aga gtg gga gaa tat atc tta gca 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 16
LENGTH: 196
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 16
Met Ile Tyr Lys Glu Lys Leu Thr Arg Val Gly Glu Tyr Ile Leu Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 17
LENGTH: 876
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(876)
SEQUENCE: 17
atg aat aaa aaa aac aag ttt att ata gct aca gca ttg gta tat tta 48. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 18
LENGTH: 291
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 18
Met Asn Lys Lys Asn Lys Phe Ile Ile Ala Thr Ala Leu Val Tyr Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 19
LENGTH: 396
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(396)
SEQUENCE: 19
tct aga ata cat gat gaa aat tat gct att aca aca aat aat aaa tta 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 20
LENGTH: 131
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 20
Ser Arg Ile His Asp Glu Asn Tyr Ala Ile Thr Thr Asn Asn Lys Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 21
LENGTH: 888
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(888)
SEQUENCE: 21
atg aca aag aaa ttt aat ttt gta aat gtt ata tta aca ttt ttg tta 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 22
LENGTH: 295
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 22
Met Thr Lys Lys Phe Asn Phe Val Asn Val Ile Leu Thr Phe Leu Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 23
LENGTH: 840
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(840)
SEQUENCE: 23
atg agc aaa aaa aag ttt att aca ata gga aca gta ctt gca tct cta 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 24
LENGTH: 279
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 24
Met Ser Lys Lys Lys Phe Ile Thr Ile Gly Thr Val Leu Ala Ser Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 25
LENGTH: 852
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(852)
SEQUENCE: 25
atg agt gct aaa aaa aag ctt ttt ata ata ggg tca gtg tta gta tgt 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 26
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 26
Met Ser Ala Lys Lys Lys Leu Phe Ile Ile Gly Ser Val Leu Val Cys
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 27
LENGTH: 828
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(828)
SEQUENCE: 27
atg agt aaa aaa aat ttt att aca ata gga gca aca ctt att cat atg 48. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 28
LENGTH: 275
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 28
Met Ser Lys Lys Asn Phe Ile Thr Ile Gly Ala Thr Leu Ile His Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 29
LENGTH: 858
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(858)
SEQUENCE: 29
atg aat aat aga aaa agt ttt ttt ata ata ggt gca tca tta cta gca 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 30
LENGTH: 285
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 30
Met Asn Asn Arg Lys Ser Phe Phe Ile Ile Gly Ala Ser Leu Leu Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 31
LENGTH: 867
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(867)
SEQUENCE: 31
atg aat tgc aaa aga ttt ttc ata gca agt gca ttg ata tca cta atg 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 32
LENGTH: 288
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 32
Met Asn Cys Lys Arg Phe Phe Ile Ala Ser Ala Leu Ile Ser Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 33
LENGTH: 864
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(864)
SEQUENCE: 33
atg aaa tat aaa aaa act ttt aca gta act gca tta gta tta tta act 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 34
LENGTH: 287
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 34
Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu Val Leu Leu Thr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 35
LENGTH: 924
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(924)
SEQUENCE: 35
atg ttt tat act aat ata tat att ctg gct tgt att tac ttt gca ctt 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 36
LENGTH: 307
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 36
Met Phe Tyr Thr Asn Ile Tyr Ile Leu Ala Cys Ile Tyr Phe Ala Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 37
LENGTH: 843
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(843)
SEQUENCE: 37
atg aat tgc aaa aaa att ctt ata aca act gca tta atg tca tta atg 48. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 38
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 38
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Ala Leu Met Ser Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 39
LENGTH: 852
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(852)
SEQUENCE: 39
atg aat tgt aaa aaa gtt ttc aca ata agt gca ttg ata tca tcc ata 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 40
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 40
Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala Leu Ile Ser Ser Ile
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 41
LENGTH: 831
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(831)
SEQUENCE: 41
atg aac tgt aaa aaa ttt ctt ata aca act aca ttg gta tca cta aca 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 42
LENGTH: 276
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 42
Met Asn Cys Lys Lys Phe Leu Ile Thr Thr Thr Leu Val Ser Leu Thr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 43
LENGTH: 882
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(882)
SEQUENCE: 43
atg aat aat aaa ctc aaa ttt act ata ata aac aca gta tta gta tgc 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 44
LENGTH: 293
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 44
Met Asn Asn Lys Leu Lys Phe Thr Ile Ile Asn Thr Val Leu Val Cys
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 45
LENGTH: 900
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(900)
SEQUENCE: 45
atg aat agc aag agt aag ttc ttt aca ata tgt aca tcg tta ata tgc 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 46
LENGTH: 299
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 46
Met Asn Ser Lys Ser Lys Phe Phe Thr Ile Cys Thr Ser Leu Ile Cys
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 47
LENGTH: 843
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(843)
SEQUENCE: 47
atg aat tat aag aaa att cta gta aga agc gcg tta atc tca tta atg 48. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 48
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 48
Met Asn Tyr Lys Lys Ile Leu Val Arg Ser Ala Leu Ile Ser Leu Met
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 53
LENGTH: 882
TYPE: DNA
ORGANISM: ***p30*** -6
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(882)
SEQUENCE: 53
atg gca aat ttt atg tac aaa aaa tac aaa cta atg aca gca ggt gta 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 54
LENGTH: 293
TYPE: PRT
ORGANISM: ***p30*** -6
SEQUENCE: 54
Met Ala Asn Phe Met Tyr Lys Lys Tyr Lys Leu Met Thr Ala Gly Val
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 55
LENGTH: 891
TYPE: DNA
ORGANISM: ***p30*** -7
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(891)
SEQUENCE: 55
atg gga aat tct atg aat aat aaa agt caa ttc tta ata aga ttt ata 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 56
LENGTH: 296
TYPE: PRT
ORGANISM: ***p30*** -7
SEQUENCE: 56
Met Gly Asn Ser Met Asn Asn Lys Ser Gln Phe Leu Ile Arg Phe Ile
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 57
LENGTH: 846
TYPE: DNA
ORGANISM: ***p30*** -9
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(846)
SEQUENCE: 57
atg aat aat aaa aga aat ttt ttt tta ata ggt atg tct cta ttg ata 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 58
LENGTH: 281
TYPE: PRT
ORGANISM: ***p30*** -9
SEQUENCE: 58
Met Asn Asn Lys Arg Asn Phe Phe Leu Ile Gly Met Ser Leu Leu Ile
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 59
LENGTH: 840
TYPE: DNA
ORGANISM: ***p30*** -11
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(840)
SEQUENCE: 59
atg aac aaa aag aaa att att aca gta gga aca aca tta gct tat tta 48. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 60
LENGTH: 279
TYPE: PRT
ORGANISM: ***p30*** -11
SEQUENCE: 60
Met Asn Lys Lys Lys Ile Ile Thr Val Gly Thr Thr Leu Ala Tyr Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 61
LENGTH: 726
TYPE: DNA
ORGANISM: ***p30*** -12
FEATURE:
NAME/KEY: CDS
LOCATION: (1)..(726)
SEQUENCE: 61
ccc gtc gtt tct cat tac agt gac ttt tca att aaa gaa act tat act 48. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 62
LENGTH: 241
TYPE: PRT
ORGANISM: ***p30*** -12
SEQUENCE: 62
Pro Val Val Ser His Tyr Ser Asp Phe Ser Ile Lys Glu Thr Tyr Thr
1 5. . .

CLM What is claimed is:

1. An isolated polynucleotide encoding an outer membrane protein of E. ***canis*** , a variant of said outer membrane protein, or an antigenic fragment of said protein; wherein the outer membrane protein is selected from the group consisting of ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12.

3. The isolated polynucleotide of claim 1 wherein said polynucleotide encodes the ***P30*** protein, a variant of the ***P30*** protein or an antigenic fragment of said ***P30*** protein.

6. An isolated polynucleotide encoding an outer membrane protein of E. ***chaffeensis*** , a variant of said outer membrane protein, or a an antigenic fragment of said outer membrane protein, wherein the outer. .

9. An isolated polypeptide selected from the group consisting of the ***P30*** protein, a variant of the ***P30*** protein, an antigenic fragment of the ***P30*** protein, the ***P30a*** protein, a variant of the ***P30a*** protein, the ***P30*** -1 protein, a variant of the ***p30*** -1 protein, the ***P30*** -2 protein, a variant of the ***P30*** -2 protein, the ***P30*** -3 protein, a variant of the ***P30*** -3 protein, the ***P30*** -4 protein, a variant of the ***P30*** -4 protein, the ***P30*** -5 protein, a variant of the ***P30*** -5 protein, the ***P30*** -6 protein, a variant of the ***P30*** -6 protein, the ***P30*** -7 protein, a variant of the ***P30*** -7 protein, the ***P30*** -8 protein, a variant of the ***P30*** -8 protein, the ***P30*** -9 protein, a variant of the ***P30*** -9 protein, the ***P30*** -10 protein, a variant of the ***P30*** -10 protein, a ***P30*** -11 protein, a variant of the ***P30*** -11 protein, the P20-12 protein, and a variant of the ***P30*** -12 protein.

11. The isolated polypeptide of claim 9 wherein said polypeptide is the ***P30*** protein, a variant of the ***P30*** protein, or an antigenic fragment of the ***P30*** protein.

15. A method for diagnosing an infection with E. ***chaffeensis*** in a patient comprising the steps of: (a) providing a serum sample from the patient; (b) providing a polypeptide selected. . . between antibodies in the serum sample and the polypeptide, wherein formation of said complex is indicative of infection with E. ***chaffeensis*** .

16. The method of claim 5 wherein said polypeptide is the ***P30*** protein, a variant of the ***P30*** protein, or an antigenic fragment of the ***P30*** protein.

19. A method for diagnosing an infection with E. ***canis*** in a Canidae patient comprising the steps of: (a) providing a serum sample from the patient; (b) providing a polypeptide. . . between antibodies in the serum sample and the polypeptide, wherein formation of said complex is indicative of infection with E. ***canis*** .

20. An antibody which binds to a protein selected from the group consisting of ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, ***P30*** -12, OMP-1, OMP-1A, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y, OMP-1Z, OMP-1H and combinations thereof.

21. A kit for diagnosing E. ***chaffeensis*** in a patient, said kit comprising a reagent selected from the group consisting of: the polypeptide of claim 3, the ***P30*** protein, a variant of the ***P30*** protein, an antigenic fragment of the ***P30*** protein, and combinations thereof.

L7 ANSWER 3 OF 30 USPATFULL
AN 2003:140409 USPATFULL
TI Homologous 28-kilodalton immunodominant protein genes of ***Ehrlichia*** ***canis*** and uses thereof
IN Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Houston, TX, UNITED STATES
McBride, Jere W., Galveston, TX, UNITED STATES
PI US 2003096250 A1 20030522
AI US 2002-62920 A1 20020131 (10)
RLI Division of Ser. No. US 2000-660587, filed on 12 Sep 2000, GRANTED, Pat. No. US 6392023 Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999, GRANTED, Pat. No. US 6403780 Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998, GRANTED, Pat. No. US 6458942
DT Utility

FS APPLICATION
LREP Benjamin Aaron Adler, Ph.D., J.D., Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 20 Drawing Page(s)
LN.CNT 2208
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.
TI Homologous 28-kilodalton immunodominant protein genes of ***Ehrlichia*** ***canis*** and uses thereof
AB . . . of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and. . .
SUMM . . . of molecular biology. More specifically, the present invention relates to molecular cloning and characterization of homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , a multigene locus encoding the 28-kDa homologous proteins of ***Ehrlichia*** ***canis*** and uses thereof.
SUMM [0005] Canine ***ehrlichiosis*** , also known as canine tropical pancytopenia, is a tick-borne rickettsial disease of dogs first described in Africa in 1935 and. . .
SUMM [0006] The etiologic agent of canine ***ehrlichiosis*** is ***Ehrlichia*** ***canis*** , a small, gram-negative, obligate intracellular bacterium which exhibits tropism for mononuclear phagocytes (Nyindo et al., 1971) and is transmitted by the brown dog tick, Rhipicephalus sanguineus (Groves et al., 1975). The progression of canine ***ehrlichiosis*** occurs in three phases, acute, subclinical and chronic. The acute phase is characterized by fever, anorexia, depression, lymphadenopathy and mild. . .

SUMM . . . persistent infections in the host. Although disease pathogenesis is poorly understood, multigene families described in members of the related genera ***Ehrlichia*** , Anaplasma, and Cowdria may be involved in variation of major surface antigen expression thereby evading immune surveillance. Anaplasma marginale, an organism closely related to E. ***canis*** , exhibits variation of major surface protein 3 (msp-3) genes resulting in antigenic polymorphism among strains (Alleman et al., 1997).

SUMM [0008] Molecular taxonomic analysis based on the 16S rRNA gene has determined that E. ***canis*** and E. ***chaffeensis*** , the etiologic agent of human monocytic ***ehrlichiosis*** (HME), are closely related (Anderson et al., 1991; Anderson et al., 1992; Dawson et al., 1991; Chen et al., 1994). Considerable cross reactivity of the 64, 47, 40, 30, 29 and 23-kDa antigens between E. ***canis*** and E. ***chaffeensis*** has been reported (Chen et al., 1994; Chen et al., 1997; Rikihisa et al., 1994; Rikihisa et al., 1992). Analysis. . . with human and canine convalescent phase sera by immunoblot has resulted in the identification of numerous immunodominant proteins of E. ***canis*** , including a 30-kDa protein (Chen et al., 1997). In addition, a 30-kDa protein of E. ***canis*** has been described as a major immunodominant antigen recognized early in the immune response that is antigenically distinct from the 30-kDa protein of E. ***chaffeensis*** (Rikihisa et al., 1992; Rikihisa et al., 1994). Other immunodominant proteins of E. ***canis*** with molecular masses ranging from 20 to 30-kDa have also been identified (Brouqui et al., 1992; Nyindo et al., 1991;. . .

SUMM [0009] Homologous 28-32 kDa immunodominant proteins encoded by multigene families have been reported in related organisms including, E. ***chaffeensis*** and Cowdria ruminantium (Sulsona et al., 1999; Ohashi et al., 1998a; Reddy et al., 1998). Recently, characterization of a 21 member multigene family encoding proteins of 23 to 28-kDa has been described in E. ***chaffeensis*** (Yu et al., 2000). The E. ***chaffeensis*** 28-kDa outer membrane proteins are surface exposed, and contain three major hypervariable regions (Ohashi et al., 1998a). The recombinant E. ***chaffeensis*** P28 appeared to provide protection against homologous challenge infection in mice, and antisera produced against the recombinant protein cross reacted with a 30-kDa protein of E. ***canis*** (Ohashi et al., 1998a). Diversity in the p28 gene among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a), and studies using monoclonal antibodies have further demonstrated diversity in the expressed P28 proteins (Yu et al., 1993). Conversely, complete conservation of a p28 genes in geographically different isolates of E. ***canis*** has been reported and suggests that E. ***canis*** may be conserved in North America (McBride et al., 1999, 2000).

SUMM . . . The prior art is deficient in the lack of cloning and characterization of new homologous 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** and a single multigene locus containing the homologous 28-kDa protein genes. Further, The prior art is deficient in the lack of recombinant proteins of such immunoreactive genes of ***Ehrlichia*** ***canis*** . The present invention fulfills this long-standing need and desire in the art.

SUMM . . . of the present invention describe the molecular cloning, sequencing, characterization, and expression of homologous mature 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** (designated p28-1, -2, -3, -5, -6, -7, -9), and the identification of a single locus (10,677-bp) containing nine 28-kDa protein genes of ***Ehrlichia*** ***canis*** (p28-1 to p28-9). Eight of the p28 genes were located on one DNA strand, and one p28 gene was found. . .

SUMM [0012] In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

SUMM . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

SUMM [0016] The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, e.g., the stimulation of the subject's humoral or cellular immune. . .

DRWD . . . arrow) and 16-kDa thioredoxin control (Lane 2, arrow), and corresponding immunoblot of recombinant p28-7-thioredoxin fusion protein recognized by covalescent-phase E. ***canis*** canine antiserum (Lane 3). Thiroredoxin control was not detected by E. ***canis*** antiserum (not shown).

DRWD . . . ID NO. 2), p28-5 protein (ECa28SA2, partial sequence, SEQ ID NO. 7), p28-4 protein (ECa28SA1, SEQ ID NO. 8), E. ***chaffeensis*** P28 (SEQ ID NO. 9), E. ***chaffeensis*** OMP-1 family (SEQ ID NOs: 10-14) and C. ruminantium MAP-1 protein (SEQ ID NO. 15). The p28-7 amino acid sequence. . .

DRWD [0022] FIG. 4 shows phylogenetic relatedness of E. ***canis*** p28-7

(ECa28-1), p28-5 (ECa28SA2, partial sequence), p28-4 (ECa28SA1), members of the E. ***chaffeensis*** omp-1 multiple gene family, and C. rumanintium map-1 protein from deduced amino acid sequences utilizing unbalanced tree construction. The length. . .

DRWD [0023] FIG. 5 shows Southern blot analysis of E. ***canis*** genomic DNA completely digested with six individual restriction enzymes and hybridized with a p28-7 DIG-labeled probe (Lanes 2-7); DIG-labeled molecular. . .

DRWD [0024] FIG. 6 shows comparison of predicted protein characteristics of E. ***canis*** p28-7 (ECa28-1, Jake strain) and E. ***chaffeensis*** P28 (Arkansas strain). Surface probability predicts the surface residues by using a window of hexapeptide. A surface residue is any. . .

DRWD [0025] FIG. 7 shows nucleic acid sequences and deduced amino acid sequences of the E. ***canis*** 28-kDa protein genes p28-5 (nucleotide 1-849: SEQ ID No. 3; amino acid sequence: SEQ ID No. 4) and p28-6 (nucleotide. . .

DRWD [0026] FIG. 8 shows schematic of the E. ***canis*** 28-kDa protein gene locus (5.592-Kb, containing five genes) indicating genomic orientation and intergenic noncoding regions (28NC1-4). The 28 -kDa protein. . .

DRWD [0027] FIG. 9 shows phylogenetic relatedness of the E. ***canis*** 28-kDa protein gene p28-4 (ECa28SA1), p28-5 (ECa28SA2), p28-6 (ECa28SA3), p28-7 (ECa28-1) and p28-8 (ECa28-2) based on amino acid sequences utilizing. . .

DRWD [0028] FIG. 10 shows alignment of E. ***canis*** 28-kDa protein gene intergenic noncoding nucleic acid sequences (SEQ ID Nos. 30-33). Nucleic acids not shown, denoted with a dot. . .

DRWD [0029] FIG. 11 shows schematic representation of the nine gene E. ***canis*** p28 locus (10,677-bp) indicating genomic orientation and intergenic noncoding regions. The p28 genes (p28-1, 2, 3, 9) (unshaded) were identified in Example 8. Shaded p28 genes have been identified previously and designated as follows: p28-4, ***p30a*** (Ohashi et al., 1998b) and ORF1 (Reddy et al., 1998); p28-5 and p28-6, (McBride, et. al., 2000); p28-7, p28 (McBride et al., 1999) and ***p30*** (Ohashi et al., 1998b); and p28-8, ***p30*** -1 (Ohashi et al., 1998b).

DRWD [0030] FIG. 12 shows phylogenetic relationships of E. ***canis*** P28-1 to P28-9 based on the amino acid sequences. The length of each pair of branches represents the distance between. . .

DRWD . . . 13 shows nucleic acid sequence (SEQ ID No. 39) and deduced amino acid sequence (SEQ ID No. 40) of E. ***canis*** p28-1 gene.

DRWD . . . shows nucleic acid sequence (SEQ ID No. 41) and deduced amino acid sequence (SEQ ID No. 42) of E. ***canis*** p28-2 gene.

DRWD . . . 15 shows nucleic acid sequence (SEQ ID No. 43) and deduced

amino acid sequence (SEQ ID No. 44) of E. ***canis*** p28-3 gene.

DRWD . . . 16 shows nucleic acid sequence (SEQ ID No. 45) and deduced amino acid sequence (SEQ ID No. 46) of E. ***canis*** p28-9 gene.

DETD [0035] The present invention describes cloning, sequencing and express ion of homologous genes encoding a 30-kilodalton (kDa) protein of ***Ehrlichia*** ***canis*** . A comparative molecular analysis of homologous genes among seven E. ***canis*** isolates and the E. ***chaffeensis*** omp-1 multigene family was also performed. Several new 28-kDa protein genes are identified as follows:

DETD [0038] Using PCR to amplify 28-kDa protein genes of E. ***canis*** , a previously unsequenced region of p28-5 (Eca28SA2) was completed. Sequence analysis of p28-5 revealed an 849-bp open reading frame encoding. . .

DETD . . . of tandemly arranged p28 genes were sequenced, and p28-1, -2, -3, and -9 were identified. Consequently, a nine gene E. ***canis*** p28 locus spanning 10, 677 bp was identified in the present invention.

DETD [0041] The present invention is directed to, inter alia, homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , p28-1, -2, -3, -6, -7, and p28-9, and a complete sequence of previously partially sequenced p28-5. Also disclosed is a multigene locus encoding nine homologous 28-kDa outer membrane proteins of ***Ehrlichia*** ***canis*** . Eight of the p28 genes were located on one DNA strand, and one p28 gene was found on the complementary. . .

DETD [0043] The invention includes a substantially pure DNA encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably. . .

DETD . . . listed in SEQ ID No 1, 3, 5, 39, 41, 43, or 45 which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** .

DETD . . . comprises a vector comprising a DNA sequence coding for a which encodes a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and said vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication;. . .

DETD . . . or viral nucleic acid. Vectors may be used to amplify and/or express nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to. . .

DETD . . . such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention can be used to transform a host using any of the techniques commonly known to those. . . Especially preferred is the use of a vector containing coding sequences for a gene encoding a 28-kDa immunoreactive protein of

Ehrlichia ***canis*** of the present invention for purposes of prokaryote transformation.

DETD . . . "recombinant" cell is intended to refer to a cell into which a recombinant gene, such as a gene encoding an ***Ehrlichia*** ***canis*** antigen has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced. . .

DETD [0053] The present invention is also drawn to substantially pure 28-30 kDa immunoreactive proteins of E. ***canis*** comprise of amino acid sequences listed in, for example, SEQ ID No. 2, 4, 6, 40, 42, 44, or 46.

DETD . . . more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** ; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography such as immunoaffinity chromatography using a n antibody specific for a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from. . .

DETD . . . In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (SEQ ID No. 2, 4, 6, 40, 42, 44, or 46). As used herein, "fragment," as applied to a polypeptide,. . . 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , by recombinant DNA techniques using an expression vector that encodes a defined fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (e.g., binding to an antibody specific for 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis***) can be assessed by methods described herein.

DETD [0056] Purified 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** or antigenic fragments of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay). . .

DETD [0059] Included in this invention are polyclonal antisera generated by

using 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** or a fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant ***Ehrlichia*** ***canis*** cDNA clones, and to distinguish them from known cDNA clones.

DETD [0067] In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

DETD . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

DETD [0071] The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, i.e. the stimulation of the subject's humoral or cellular immune. . .

DETD [0074] ***Ehrlichiae*** and Purification ***Ehrlichia*** ***canis*** (Florida strain and isolates Demon, D J, Jake, and Fuzzy) were provided by Dr. Edward Breitschwerdt, (College of Veterinary Medicine, North Carolina State University, Raleigh, N.C.). E. ***canis*** (Louisiana strain) was provided by Dr. Richard E. Corstvet (School of Veterinary Medicine, Louisiana State University, Baton Rouge, La.) and E. ***canis*** (Oklahoma strain) was provided by Dr. Jacqueline Dawson (Centers for Disease Control and Prevention, Atlanta, Ga.). Propagation of ***ehrlichiae*** was performed in DH82 cells with DMEM supplemented with 10% bovine calf serum and 2 mM L-glutamine at 37.degree. C. The intracellular growth in DH82 cells was monitored by presence of E. ***canis*** morulae using general cytologic staining methods. Cells were harvested when 100% of the cells were infected with ***ehrlichiae*** and were then pelleted in a centrifuge at 17,000.times.g for 20 min. Cell pellets were disrupted with a Braun-Sonic 2000 sonicator twice at 40W for 30 sec on ice. ***Ehrlichiae*** were purified as described previously (Weiss et al.,

1975). The lysate was loaded onto discontinuous gradients of 42%-36%-30% renografin, and centrifuged at 80,000.times.g for 1 hr. Heavy and light bands containing ***ehrlichiae*** were collected and washed with sucrose-phosphate-glutamate buffer (SPG, 218 mM sucrose, 3.8 mM KH.sub.2PO.sub.4, 7.2 mM K.sub.2HPO.sub.4, 4.9 mM glutamate,. . .

DETD [0075] Nucleic Acid Preparation ***Ehrlichia*** ***canis*** genomic DNA was prepared by resuspending the renografin-purified ***ehrlichiae*** in 600 .mu.l of 10 mM Tris-HCl buffer (pH 7.5) with 1% sodium dodecyl sulfate (SDS, w/v) and 100 ng/ml. . .

DETD . . . determined using a Universal GenomeWalker Kit (CLONTECH, Palo Alto, Calif.) according to the protocol supplied by the manufacturer. Genomic E. ***canis*** (Jake isolate) DNA was digested completely with five restriction enzymes (DraI, EcoRV, PvuII, ScaI, StuI) which produce blunt-ended DNA. An adapter (AP1) supplied in the kit was ligated to each end of E. ***canis*** DNA. The genomic libraries were used as templates to find the unknown DNA sequence of the p28-7 gene by PCR. . .

DETD . . . with an ABI Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The entire p28-7 genes of seven E. ***canis*** isolates (four from North Carolina, and one each from Oklahoma, Florida, and Louisiana) were amplified by PCR with primers EC28OM-F. . .

DETD PCR Amplification, Cloning, Sequencing and Expression of E. ***canis*** ECa28-1 (p28-7) Gene

DETD [0078] Expression Vectors The entire E. ***canis*** p28-7 gene was PCR-amplified with primers-EC28OM-F and EC28OM-R and cloned into pCR2.1-TOPO TA cloning vector to obtain the desired set. . .

DETD [0079] Western Blot Analysis Recombinant E. ***canis*** p28-7 fusion protein was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-15% Tris-HCl gradient gels (Bio-Rad, Hercules, Calif.) and transferred. . . N.H.) using a semi-dry transfer cell (Bio-Rad, Hercules, Calif.). The membrane was incubated with convalescent phase antisera from an E. ***canis*** -infected dog diluted 1:5000 for 1 hour, washed, and then incubated with an anti-canine IgG (H &L) alkaline phosphatase-conjugated affinity-purified. . .

DETD [0080] Southern Blot Analysis To determine if multiple genes homologous to the p28-7 gene were present in the E. ***canis*** genome, a genomic Southern blot analysis was performed using a standard procedure (Sambrook et al. 1989). E. ***canis*** genomic DNA digested completely with each of the restriction enzymes BanII, EcoRV, HaeII, KpnI and SpeI, which do not cut. . . digested probe (566-bp) was separated by agarose gel electrophoresis, gel-purified and then used for hybridization. The completely digested genomic E. ***canis*** DNA was electrophoresed and transferred to a nylon membrane (Boehringer Mannheim, Indianapolis, Ind.) and hybridized at 40.degree. C. for 16. .

DETD [0081] Sequence Analysis and Comparasion E. ***chaffeensis*** p28 and C. ruminantium map-1 DNA sequences were obtained from the National Center of Biotechnology Information (NCBI). Nucleotide and deduced. .

DETD [0082] Sequence analysis of p28-7 from seven different strains of E. ***canis*** was performed with primers designed to amplify the entire gene. Analysis revealed the sequence of this gene was conserved among.

DETD [0084] Alignment of nucleic acid sequences from E. ***chaffeensis*** p28 and Cowdria ruminantium map-1 using the Jotun-Hein aligorithm produced a consensus sequence with regions of high homology (>90%). These homologous regions (nucleotides 313-332 and 823-843 of C. ruminantium map-1; 307-326 and 814-834 of E. ***chaffeensis*** p28) were targeted as primer annealing sites for PCR amplification. PCR amplification of the E. ***canis*** p28-7 gene was accomplished with primers 793 (5-GCAGGAGCTGTTGGTTACTC-3') (SEQ ID NO. 16) and 1330 (5'-CCTTCCTCCAAGTTCTATGCC-3') (SEQ ID NO. 17), resulting in a 518-bp PCR product. E. ***canis*** DNA was amplified with primers 793 and 1330 with a thermal cycling profile of 95.degree. C. for 2 min, and. . . followed by a 72.degree. C. extension for 10 min and 4.degree. C. hold. The nucleic acid sequence of the E. ***canis*** PCR product was obtained by sequencing the product directly with primers 793 and 1330.

DETD . . . frame encoding a protein of 170 amino acids, and alignment of the 518-bp sequence obtained from PCR amplification of E. ***canis*** with the DNA sequence of E. ***chaffeensis*** p28 gene revealed a similarity greater than 70%, indicating that the genes were homologous.

DETD . . . PCR product amplified with these primers was sequenced directly with the same primers. The complete DNA sequence for the E. ***canis*** p28-7 gene (SEQ ID NO. 1) is shown in FIG. 1. The p28-7 PCR fragment amplified with these primers contained. . . amino acids from the multiple cloning site and 5' non-coding primer region at the N-terminus. Convalescent-phase antiserum from an E. ***canis*** infected dog recognized the expressed recombinant fusion protein, but did not react with the thioredoxin control (FIG. 2).

DETD [0089] Sequence Homology of E. ***canis*** p28-7 Gene

DETD [0090] The nucleic acid sequence of E. ***canis*** p28-7 (834-bp) and the E. ***chaffeensis*** omp-1 family of genes including signal sequences (p28-7, omp-1A, B, C, D, E, and F) were aligned using the Clustal method to examine homology between these genes (alignment not shown). Nucleic acid homology was equally conserved (68.9%) between E. ***canis*** p28-7, E. ***chaffeensis*** p28 and omp-1F. Other putative outer membrane protein genes in the E. ***chaffeensis*** omp-1 family, omp-1D (68.2%), omp-1E (66.7%), omp-1C (64.1%), Cowdria ruminantium map-1 (61.8%), E. ***canis*** 28-kDa protein 1 gene

(60%) and 28-kDa protein 2 gene (partial) (59.5%) were also homologous to p28-7. E. ***chaffeensis*** omp-1B had the least nucleic acid homology (45.1%) with E. ***canis*** p28-7.

DETD [0091] Alignment of the predicted amino acid sequences of E. ***canis*** P28-7 (SEQ ID NO. 2) and E. ***chaffeensis*** P28 revealed amino acid substitutions resulting in four variable regions (VR). Substitutions or deletions in the amino acid sequence and the locations of variable regions of E. ***canis*** P28-7 and the E. ***chaffeensis*** OMP-1 family were identified (FIG. 3). Amino acid comparison including the signal peptide revealed that E. ***canis*** P28-7 shared the most homology with OMP-IF (68%) of the E. ***chaffeensis*** OMP-1 family, followed by E. ***chaffeensis*** P28 (65.5%), OMP-1E (65.1%), OMP-1D (62.9%), OMP-1C (62.9%), Cowdria ruminantium MAP-1 (59.4%), E. ***canis*** 28-kDa protein 1 (55.6%) and 28-kDa protein 2 (partial) (53.6%), and OMP-1B (43.2%). The phylogenetic relationships based on amino acid sequences show that E. ***canis*** P28-7 and C. ruminantium MAP-1, E. ***chaffeensis*** OMP-1 proteins, and E. ***canis*** 28-kDa proteins 1 and 2 (partial) are related (FIG. 4).

DETD [0092] Predicted Surface Probability and Immunoreactivity of E. ***canis*** P28-7

DETD [0093] Analysis of E. ***canis*** P28-7 using hydropathy and hydrophilicity profiles predicted surface-exposed regions on P28-7 (FIG. 6). Eight major surface-exposed regions consisting of 3 to 9 amino acids were identified on E. ***canis*** P28-7 and were similar to the profile of surface-exposed regions on E. ***chaffeensis*** P28 (FIG. 6). Five of the larger surface-exposed regions on E. ***canis*** P28-7 were located in the N-terminal region of the protein. Surface-exposed hydrophilic regions were found in all four of the variable regions of E. ***canis*** P28-7. Ten T-cell motifs were predicted in the P28-7 using the Rothbard-Taylor aligorithm (Rothbard and Taylor, 1988), and high antigenicity of the E. ***canis*** P28-7 was predicted by the Jameson-Wolf antigenicity aligorithm (FIG. 6) (Jameson and Wolf, 1988). Similarities in antigenicity and T-cell motifs were observed between E. ***canis*** P28-7 and E. ***chaffeensis*** P28.

DETD [0094] Detection of Homologous Genomic Copies of E. ***canis*** p28-7 Gene

DETD [0095] Genomic Southern blot analysis of E. ***canis***. DNA completely digested independently with restriction enzymes BanII, EcoRV, HaeII, KpnI, SpeI, which do not have restriction endonuclease sites in. . . nucleotides 34, 43 and 656, revealed the presence of at least three homologous p28-7 gene copies (FIG. 5). Although E. ***canis*** p28-7 has internal Ase I internal restriction sites, the DIG-labeled probe used in the hybridization experiment targeted a region of. . .

DETD [0096] PCR Amplification of E. ***canis*** ECa28SA2 (p28-5), ECa28SA3 (p28-6)

DETD [0102] Nucleic and Amino Acid Homology of E. ***canis*** p28-4, p29-5, p28-6, p28-7 and p28-9 proteins

DETD [0103] The nucleic and amino acid sequences of all five E. ***canis*** 28-kDa protein genes were aligned using the Clustal method to examine the homology between these genes. The nucleic acid homology. . . 58 to 75% and a similar amino acid homology of ranging from 67 to 72% was observed between the E. ***canis*** 28-kDa protein gene members (FIG. 9).

DETD . . . protein genes were analyzed for promoter sequences by comparison with consensus Escherichia coli promoter regions and a promoter from E. ***chaffeensis*** (Yu et al., 1997; McClure, 1985). Putative promoter sequences including RBS, -10 and -35 regions were identified in 4 intergenic. . .

DETD [0105] N-Terminal Signal Sequence The amino acid sequence analysis revealed that entire E. ***canis*** p28-7 has a deduced molecular mass of 30.5-kDa and the entire p28-6 has a deduced molecular mass of 30.7-kDa. Both. . . N-terminal signal peptide of 23 amino acids (MNCKKILITTALMSLMYYAPSIS, SEQ ID No. 27), which is similar to that predicted for E. ***chaffeensis*** P28 (MNYKKILITSALISLISSLPGV SFS, SEQ ID NO. 28), and the OMP-1 protein family (Yu et al., 1999a; Ohashi et al., 1998b).

DETD . . . cleavage site at amino acid position 25 (MNCKKILITTALISLMYSIPSISSFS, SEQ ID NO. 29) identical to the predicted cleavage site of E. ***chaffeensis*** P28 (SFS) was also present, and would result in a mature p28-7 with a predicted molecular mass of 27.7-kDa. Signal. . .

DETD [0108] Proteins of similar molecular mass have been identified and cloned from multiple rickettsial agents including E. ***canis*** , E. ***chaffeensis*** , and C. ruminantium (Reddy et al., 1998; Jongejan et al., 1993; Ohashi et al., 1998). A single locus in ***Ehrlichia*** ***chaffeensis*** with 6 homologous p28 genes, and 2 loci in E. ***canis*** , each containing some homologous 28-kDa protein genes have been previously described.

DETD [0109] The present invention demonstrated the cloning, expression and characterization of genes encoding mature 28-kDa proteins of E. ***canis*** that are homologous to the omp-1 multiple gene family of E. ***chaffeensis*** and the C. ruminantium map-1 gene. Two new 28-kDa protein genes were identidfied, p28-7 and p28-6. Another E. ***canis*** 28-kDa protein gene, p28-5, partially sequenced previously (Reddy et al., 1998), was sequenced completely in the present invention. Also disclosed is the identification and characterization of a single locus in E. ***canis*** containing five E. ***canis*** 28-kDa protein genes (p28-4, p28-5, p28-6, p28-7 and p28-8).

DETD [0110] The E. ***canis*** 28-kDa proteins are homologous to E. ***chaffeensis*** OMP-1 family and the MAP-1 protein of C. rumanintium. The most homologous E. ***canis*** 28-kDa proteins (p28-6, p28-7 and p28-8) are sequentially arranged in the locus. Homology of these proteins ranged from 67.5% to 72.3%. Divergence among these 28-kDa proteins was 27.3% to 38.6%. E. ***canis*** 28-kDa proteins p28-4 and p28-5 were the least homologous with homology ranging from 50.9% to 59.4% and divergence of 53.3. . . these regions are surface exposed and subject to selective pressure by the immune system. Conservation of p28-7 among seven E. ***canis*** isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be clonal in North America. Conversely, significant diversity of p28 among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a).

DETD [0111] All of the E. ***canis*** 28-kDa proteins appear to be post translationally processed from a 30-kD protein to a mature 28-kD protein. Recently, a signal sequence was identified on E. ***chaffeensis*** P28 (Yu et al., 1999a), and N-terminal amino acid sequencing has verified that the protein is post-translationally processed resulting in. . . OMP-1F and OMP-1E have also been proposed as leader signal peptides (Ohashi et al., 1998). Signal sequences identified on E. ***chaffeensis*** OMP-1F, OMP-1E and P28 are homologous to the leader sequence of E. ***canis*** 28-kDa protein. Promoter sequences for the p28 genes have not been determined experimentally, but putative promoter regions were identified by comparison with consensus sequences of the RBS, -10 and -35 promoter regions of E. coli and other ***ehrlichiae*** (Yu et al., 1997; McClure, 1985). Such promoter sequences would allow each gene to potentially be transcribed and translated, suggesting. . .

DETD [0112] The E. ***canis*** 28-kda protein genes were found to exhibit nucleic acid and amino acid sequence homology with the E. ***chaffeensis*** omp-1 gene family and C. ruminantium map-1 gene. Previous studies have identified a 30-kDa protein of E. ***canis*** that reacts with convalescent phase antisera against E. ***chaffeensis*** , but was believed to be antigenically distinct (Rikihisa et al., 1994). Findings based on comparison of amino acid substitutions in four variable regions of E. ***canis*** 28-kDa proteins support this possibility. Together these findings also suggest that the amino acids responsible for the antigenic differences between E. ***canis*** and E. ***chaffeensis*** P28 are located in these variable regions and are readily accessible to the immune system.

DETD . . . It was reported that immunoreactive peptides were located in the variable regions of the 28-kDa proteins of C. ruminantium, E. ***chaffeensis*** and E. ***canis*** (Reddy et al., 1998). Analysis of E. ***canis*** and E. ***chaffeensis*** P28 revealed

that all of the variable regions have predicted surface-exposed amino acids. A study in dogs demonstrated lack of cross protection between E. ***canis*** and E. ***chaffeensis*** (Dawson and Ewing, 1992). This observation may be related to antigenic differences in the variable regions of P28 as well as in other immunologically important antigens of these ***ehrlichial*** species. Another study found that convalescent phase human antisera from E. ***chaffeensis*** -infected patients recognized 29/28-kDa protein(s) of E. ***chaffeensis*** and also reacted with homologous proteins of E. ***canis*** (Chen et al., 1997). Homologous and crossreactive epitopes on the E. ***canis*** 28-kDa protein and E. ***chaffeensis*** P28 appear to be recognized by the immune system.

DETD [0114] E. ***canis*** 28-kDa proteins may be important immunoprotective antigens. Several reports have demonstrated that the 30-kDa antigen of E. ***canis*** exhibits strong immunoreactivity (Rikihisa et al., 1994; Rikihisa et al., 1992). Antibodies in convalescent phase antisera from humans and dogs have consistently reacted with proteins in this size range from E. ***chaffeensis*** and E. ***canis*** , suggesting that they may be important immunoprotective antigens (Rikihisa et al., 1994; Chen et al., 1994; Chen et al., 1997). In addition, antibodies to 30, 24 and 21-kDa proteins developed early in the immune response to E. ***canis*** (Rikihisa et al., 1994; Rikihisa et al., 1992), suggesting that these proteins may be especially important in the immune responses. . . Recently, a family of homologous genes encoding outer membrane proteins with molecular masses of 28-kDa have been identified in E. ***chaffeensis*** , and mice immunized with recombinant E. ***chaffeensis*** P28 appeared to have developed immunity against homologous challenge (Ohashi et al., 1998). The P28 of E. ***chaffeensis*** has been demonstrated to be present in the outer membrane, and immunoelectron microscopy has localized the P28 on the surface. . . that it may serve as an adhesin (Ohashi et al., 1998). It is likely that the 28-kDa proteins of E. ***canis*** identified in this study have the same location and possibly serve a similar function.

DETD [0115] Comparison of p28-7 from different strains of E. ***canis*** revealed that the gene is apparently completely conserved. Studies involving E. ***chaffeensis*** have demonstrated immunologic and molecular evidence of diversity. Patients infected with E. ***chaffeensis*** have variable immunoreactivity to the 29/28-kDa proteins, suggesting that there is antigenic diversity (Chen et al., 1997). Recently molecular evidence has been generated to support antigenic diversity in the p28 gene from E. ***chaffeensis*** (Yu et al., 1999a). A comparison of five E. ***chaffeensis*** isolates revealed that two isolates (Sapulpa and St. Vincent) were 100%

identical, but three others (Arkansas, Jax, 91HE17) were divergent by as much as 13.4% at the amino acid level. The conservation of E. ***canis*** p28-7 suggests that E. ***canis*** strains found in the United States may be genetically identical, and thus E. ***canis*** 28-kDa protein is an attractive vaccine candidate for canine ***ehrlichiosis*** in the United States. Further analysis of E. ***canis*** isolates outside the United States may provide information regarding the origin and evolution of E. ***canis*** . Conservation of the 28-kDa protein makes it an important potential candidate for reliable serodiagnosis of canine ***ehrlichiosis*** .

DETD [0116] The role of multiple homologous genes is not known at this point; however, persistence of E. ***canis*** infections in dogs could conceivably be related to antigenic variation due to variable expression of homologous 28-kDa protein genes, thus enabling E. ***canis*** to evade immune surveillance. Variation of msp-3 genes in A. marginale is partially responsible for variation in the MSP-3 protein, resulting in persistent infections (Alleman et al., 1997). Studies to examine 28-kDa protein gene expression by E. ***canis*** in acutely and chronically infected dogs would provide insight into the role of the 28-kDa protein gene family in persistence. . .

DETD [0117] Identification of E. ***canis*** p28-1, p28-2 p28-3 and p28-9 Genes

DETD [0119] The nucleic acid and amino acid sequences of the E. ***canis*** p28 genes were aligned using the Clustal method to examine the homology between these genes. Homology of these proteins ranged from 67.5% to 75%, and divergence among these P28 proteins was 26.9% to 38%. E. ***canis*** P28 proteins P28-1, P28-2, and P28-9 were the least homologous with the other p28 genes ranging from 37% to 49%. . . nucleic acid homology of the nine p28 genes ranged from 28 to 72%. The phylogenetic relationships based on the E. ***canis*** p28 amino acid sequences are shown in FIG. 12.

DETD . . . accession numbers. The GenBank accession numbers for the nucleic acid and amino acid sequences for the complete nine gene E. ***canis*** (Jake strain) p28 gene locus is AF082744. This accession number was originally assigned to p28-7, but has been updated with. . . p28 locus, which includes p28-7. GenBank accession numbers for nucleic acid and amino acid sequences of p28-7 in other E. ***canis*** isolates described in this study are: Louisiana, AF082745; Oklahoma, AF082746; Demon, AF082747; DJ, AF082748; Fuzzy, AF082749; Florida, AF082750.

DETD [0121] Multiple bands in the 28-kilodalton range have been observed by immunoblots of convalescent sera from E. ***canis*** infected dogs (Rikihisa et al., 1994), and expression of multiple p28 proteins could be an explanation for this observation. Southern. . .

DETD [0122] In this study a single gene locus containing nine tandemly

arranged E. ***canis*** p28 genes encoding homologous, but nonidentical, p28 genes was identified. The nine gene locus included four new p28 genes (FIGS.. . .

DETD [0123] The P28s of E. ***canis*** were found to be as closely related to 28-kilodalton proteins of other species such as E. ***chaffeensis*** as they are to themselves (McBride et al., 2000). Differences among the proteins are found primarily in several major hypervariable. . .

DETD [0124] Conservation of an E. ***canis*** p28 gene (p28-7) among seven geographically different isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be highly conserved in North America. Similarly, the 120-kDa glycoprotein of E. ***canis*** is also conserved among isolates in the United States (Yu et al., 1997). In contrast, both the 120-kDa and the 28-kDa protein genes of E. ***chaffeensis*** are divergent among isolates (Yu et al., 1999a; Chen et al., 1997). The diversity of the 28-kDa protein gene of E. ***chaffeensis*** appeared to result from point mutations in the hypervariable regions perhaps due to selective immune pressure (Yu et al., 1999a). These data suggest that E. ***canis*** may have been introduced into North America relatively recently, and this may account for the conservation that was observed among isolates. The conservation of p28 genes in E. ***canis*** isolates may provide an opportunity to develop vaccine and serodiagnostic antigens that are particularly effective for disease prevention and serodiagnosis.. . .

DETD [0154] Troy G. C., et al., (1990) Canine ***ehrlichiosis*** . In Infectious diseases of the dog and cat. Green C. E. (ed). Philidelphia: W. B. Sauders Co.

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 1

LENGTH: 1607

TYPE: DNA

ORGANISM: ***Ehrlichia*** ***canis***

FEATURE:

OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-7

SEQUENCE: 1

attttattta ttaccaatct tatataatat attaaatttc tcttacaaaa atctctaatg 60

ttttatacct aatatatata ttctggcttg tatctacttt gcacttccac tattgttaat 120

ttattttcac tattttaggt gtaatatgaa ttgcaaaaaa. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 2

LENGTH: 278

TYPE: PRT

ORGANISM: ***Ehrlichia*** ***canis***

FEATURE:

OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-7 protein

SEQUENCE: 2
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Ala Leu Ile Ser Leu
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 849
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: mat_peptide
OTHER INFORMATION: nucleic acid sequence of p28-5
SEQUENCE: 3
atgaattgta aaaaagtttt cacaataagt gcattgatat catccatata cttcctacct 60
aatgtctcat actctaaccc agtatatggt aacagtatgt atggtaattt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of p28-5 protein
SEQUENCE: 4
Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala Leu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 840
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: mat_peptide
OTHER INFORMATION: nucleic acid sequence of p28-6
SEQUENCE: 5
atgaattgca aaaaaattct tataacaact gcattaatgt cattaatgta ctatgctcca 60
agcatatctt tttctgatac tatacaagac gataacactg gtagcttcta. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of p28-6 protein
SEQUENCE: 6
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Ala Leu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7

LENGTH: 133
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: partial amino acid sequence of p28-5 protein
SEQUENCE: 7
Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 8
LENGTH: 287
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of p28-4 protien
SEQUENCE: 8
Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 9
LENGTH: 281
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** P28
SEQUENCE: 9
Met Asn Tyr Lys Lys Val Phe Ile Thr Ser Ala Leu Ile Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 10
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1B
SEQUENCE: 10
Met Asn Tyr Lys Lys Ile Phe Val Ser Ser Ala Leu Ile Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 11
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1C
SEQUENCE: 11
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Ala Leu Pro

5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 12
LENGTH: 286
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1D
SEQUENCE: 12
Met Asn Cys Glu Lys Phe Phe Ile Thr Thr Ala Leu Thr Leu Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 13
LENGTH: 278
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1E
SEQUENCE: 13
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Val Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 14
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1F
SEQUENCE: 14
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Thr Leu Val Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 19
LENGTH: 20
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
OTHER INFORMATION: primer used for sequencing 28-kDa protein genes in E. ***canis***
SEQUENCE: 19
agtgcagagt cttcggtttc 20
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 20
LENGTH: 18

TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
OTHER INFORMATION: primer used for sequencing 28-kDa protein genes in E. ***canis***
SEQUENCE: 20
gttacttgcg gaggacat 18
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 21
LENGTH: 24
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
LOCATION: nucleotides 687-710 of E. ***canis*** p28-7
OTHER INFORMATION: primer 394 for PCR
SEQUENCE: 21
gcatttccac aggatcatag gtaa 24
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 22
LENGTH: 24
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
LOCATION: nucleotides 710-687 of E. ***canis*** p28-7
OTHER INFORMATION: primer 394C for PCR
SEQUENCE: 22
ttacctatga tcctgtggaa atgc 24
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 23
LENGTH: 20
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
OTHER INFORMATION: primer 793C which anneals to a region with E. ***canis*** p28-7, used to amplify the intergenic region between gene p28-6 and p28-7
SEQUENCE: 23
gagtaaccaa cagctcctgc 20
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 27
LENGTH: 23

TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: PEPTIDE
OTHER INFORMATION: a predicted N-terminal signal peptide of p28-7 and p28-6
SEQUENCE: 27
Met Asn Cys Lys Lys Ile Leu Ile. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 28
LENGTH: 25
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of N-terminal signal peptide of E. ***chaffeensis*** P28
SEQUENCE: 28
Met Asn Tyr Lys Lys Ile Leu Ile Thr Ser Ala Leu Ile Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 29
LENGTH: 26
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of putative cleavage site of p28-7
SEQUENCE: 29
Met Asn Cys Lys Lys Ile Leu Ile Thr. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 30
LENGTH: 299
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 1 (28NC1)
SEQUENCE: 30
taatacttct attgtacatg ttaaaaatag tactagtttg cttctgtggt ttataaacgc 60
aagagagaaa tagttagtaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 31
LENGTH: 345
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 2

(28NC2)
SEQUENCE: 31
taatttcgtg gtacacatat cacgaagcta aaattgtttt tttatctctg ctgtatacaa 60
gagaaaaaat agtagtgaaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 32
LENGTH: 345
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 3
(28NC3)
SEQUENCE: 32
tgattttatt gttgccacat attaaaaatg atctaaactt gtttttatta ttgctacata 60
caaaaaaaag aaaaatagtg. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 33
LENGTH: 355
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 4
(28NC4)
SEQUENCE: 33
taattttatt gttgccacat attaaaaatg atctaaactt gtttttawta ttgctacata 60
caaaaaaaga aaaatagtgg. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 39
LENGTH: 879
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-1
SEQUENCE: 39
atgaataata aactcaaatt tactataata aacacagtat tagtatgctt attgtcatta 60
cctaatatat cttcctcaaa ggccataaac aataacgcta aaaagtacta cggattatat 120
atcagtggac aatataaacc cagtgtttct gttttcagta. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 40
LENGTH: 293
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-1 protein
SEQUENCE: 40

Met Asn Asn Lys Leu Lys Phe Thr Ile Ile Asn Thr Val Leu Val
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 41
LENGTH: 840
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-2
SEQUENCE: 41
atgaattata agaaaattct agtaagaagc gcgttaatct cattaatgtc aatcttacca 60
tatcagtctt ttgcagatcc tgtaggttca agaactaatg ataacaaaga aggcttctac 120
attagtgcaa agtacaatcc aagtatatca cactttagaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 42
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-2 protein
SEQUENCE: 42
Met Asn Tyr Lys Lys Ile Leu Val Arg Ser Ala Leu Ile Ser Leu
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 43
LENGTH: 828
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-3
SEQUENCE: 43
atgaactgta aaaaaattct tataacaact acattggtat cactaacaat tcttttacct 60
ggcatatctt tctccaaacc aatacatgaa aacaatacta caggaaactt ttacattatt 120
ggaaaatatg taccaagtat ttcacatttt gggaactttt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 44
LENGTH: 276
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-3 protein
SEQUENCE: 44
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Thr Leu Val Ser Leu
5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 45
LENGTH: 813
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-9
SEQUENCE: 45
atgaattaca aaagatttgt tgtaggtgtt acgctgagta catttgtttt tttcttatct 60
gatggtgctt tttctgatgc aaatttttct gaagggagga gaggacttta tataggtagt 120
cagtataaag ttggtattcc caattttagt aatttttcag. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 46
LENGTH: 271
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-9 protein
SEQUENCE: 46
Met Asn Tyr Lys Arg Phe Val Val Gly Val Thr Leu Ser Thr Phe
5. . .
CLM What is claimed is:

1. An isolated DNA sequence encoding a 30-kilodalton protein of ***Ehrlichia*** ***canis*** , wherein said protein is immunoreactive with anti- ***Ehrlichia*** ***canis*** serum.

6. The DNA sequence of claim 1, wherein said DNA is contained in a single locus of ***Ehrlichia*** ***canis*** .

8. The DNA sequence of claim 7, wherein said locus contains genes encoding homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** .

9. The DNA sequence of claim 8, wherein said homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** are selected from the group consisting of p28-1, p28-2, p28-3, p28-4, p28-5, p28-6, p28-7, p28-8 and p28-9.

17. A method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit ***Ehrlichia*** ***canis*** infection.

L7 ANSWER 4 OF 30 USPATFULL
AN 2003:120747 USPATFULL
TI Blood cell deficiency treatment method
IN Ahlem, Clarence N., San Diego, CA, UNITED STATES
Reading, Christopher, San Diego, CA, UNITED STATES
Frincke, James, San Diego, CA, UNITED STATES
Stickney, Dwight, Granite Bay, CA, UNITED STATES
Lardy, Henry A., Madison, WI, UNITED STATES
Marwah, Padma, Middleton, WI, UNITED STATES
Marwah, Ashok, Middleton, WI, UNITED STATES
Prendergast, Patrick T., Straffan, IRELAND
PI US 2003083231 A1 20030501
AI US 2002-87929 A1 20020301 (10)
RLI Continuation-in-part of Ser. No. US 2000-675470, filed on 28 Sep 2000, PENDING Continuation-in-part of Ser. No. US 2001-820483, filed on 29 Mar 2001, PENDING Continuation-in-part of Ser. No. US 2000-535675, filed on 23 Mar 2000, PENDING Continuation-in-part of Ser. No. US 1999-449004, filed on 24 Nov 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-449184, filed on 24 Nov 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-449042, filed on 24 Nov 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-461026, filed on 15 Dec 1999, ABANDONED Continuation-in-part of Ser. No. US 2000-586673, filed on 1 Jun 2000, ABANDONED Continuation-in-part of Ser. No. US 2000-586672, filed on 1 Jun 2000, ABANDONED Continuation-in-part of Ser. No. US 1999-414905, filed on 8 Oct 1999, ABANDONED
PRAI US 1999-161453P 19991025 (60)
US 2001-272624P 20010301 (60)
US 2001-323016P 20010911 (60)
US 2001-340045P 20011130 (60)
US 2001-328738P 20011011 (60)
US 2001-338015P 20011108 (60)
US 2001-343523P 20011220 (60)
US 1999-126056P 19991019 (60)
US 1999-124087P 19990311 (60)
US 1998-109923P 19981124 (60)
US 1998-109924P 19981124 (60)
US 1998-110127P 19981127 (60)
US 1998-112206P 19981215 (60)
US 1999-145823P 19990727 (60)
US 1999-137745P 19990603 (60)
US 1999-140028P 19990616 (60)
DT Utility
FS APPLICATION
LREP HOLLIS-EDEN PHARMACEUTICALS, INC., 4435 EASTGATE MALL, SUITE 400, SAN

DIEGO, CA, 92121
CLMN Number of Claims: 45
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 19428
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to the use of compounds to treat a number of conditions, such as thrombocytopenia, neutropenia or the delayed effects of radiation therapy. Compounds that can be used in the invention include methyl-2,3,4-trihydroxy-1-O-(7,17-dioxoandrost-5-ene-3.beta.-yl)-.beta.-D-glucopyranosiduronate, 16.alpha.,3.alpha.-dihydroxy-5.alpha.-androstan-17-one or 3,7,16,17-tetrahydroxyandrost-5-ene, 3,7,16,17-tetrahydroxyandrost-4-ene,3,7,16,17-tetrahydroxyandrost-1-ene or 3,7,16,17-tetrahydroxyandrostane that can be used in the treatment method.
SUMM . . . (e.g., L. donovani, L. major, L. braziliensis), Plasmodium sp. (e.g., P. falciparum, P. knowlesi, P. vivax, P. berghei, P. yoelli), ***Ehrlichia*** sp. (e.g., E. ***canis*** , E. ***chaffeensis*** , E. phagocytophila, E. equi, E. sennetsu), Entamoeba sp., Babesia microti, Bacillus anthracis, Brucella sp. (e.g., B. militensis, B. abortus), Bartonella. . .
SUMM . . . inducible protein B, StF-IT, NFAT, NFAT interacting protein 45 (NIP45), IkB, an IkB kinase, NFATp, NFAT4, an AP-1 family protein, ***p300*** , CREB, CREB-binding protein (CPB), ***p300*** /CBP, ***p300*** /CPB-associated factor, SWI/SNF and their human and other homologs, BRG-1, OCT-1/OAF, AP-1, AF-2, Ets, androgen receptor associated protein 54 (ARA54), androgen. . . acute regulatory protein gene promoter region, RevErb, Rev-erbA .alpha., Rev-erb .beta., steroid receptor coactivator amplified in breast cancer (AIB 1), ***p300*** /CREB binding protein-interacting protein (p/CIP), thyroid hormone receptor (TR, T3R), thyroid hormone response elements (T3REs), retinoblastoma protien (Rb), tumor suppressor factor. . . OR-1, androgen receptor, glucocorticoid receptor, estrogen receptor, progesterone receptor, mineralcorticoid receptor, aldosterone receptor, E6-associated protein (E6-AP), OR1, OR1/RXR.alpha. complex, TIF-1, CBP/ ***P300*** complex, TRIP1/SUG-1 complex, RIP-140, steroid receptor coactivator 1 (SRC1), SRC1.alpha./P160 complex and TIF-2/GRIP-1 complex, RAR/N-CoR/RIP13 complex, RAR/SMRT/TRAC-2 complex and protein. . .
SUMM . . . Exemplary transcription factors that may be present include one or more of ARA54, ARA55, ARA70, SRC-1, NF-.kappa.B, NFAT, AP1, Ets, ***p300*** , CBP, ***p300*** /CBP, ***p300*** /CPB-associated factor, SWI/SNF and human homologs of SWI/SNF, CBP, SF-1, RIP140, GRIP1 and Vpr. In general, one provides a first and. . .
SUMM . . . cell or tissue extract or the partially purified or purified cell or tissue extract. The transactivator protein may be TIF-1, CBP/

P300 , TRIP1/SUG-1, RIP-140, SRC1.alpha./P160, or TIF-2/GRIP-1. In any of these embodiments the complex comprising the steroid receptor protein, the formula 1. . .

L7 ANSWER 5 OF 30 USPATFULL
AN 2003:106184 USPATFULL
TI Homologous 28-kilodalton immunodominant protein genes of ***Ehrlichia*** ***canis*** and uses thereof
IN Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Houston, TX, UNITED STATES
McBride, Jere W., Galveston, TX, UNITED STATES
PI US 2003073095 A1 20030417
AI US 2002-62051 A1 20020131 (10)
RLI Division of Ser. No. US 2000-660587, filed on 12 Sep 2000, GRANTED, Pat. No. US 6392023 Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999, GRANTED, Pat. No. US 6403780 Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998, GRANTED, Pat. No. US 6458942
DT Utility
FS APPLICATION
LREP Benjamin Aaron Adler, Ph.D., J.D., Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 20 Drawing Page(s)
LN.CNT 2207
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5; -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.
TI Homologous 28-kilodalton immunodominant protein genes of ***Ehrlichia*** ***canis*** and uses thereof
AB . . . of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5; -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the

development of vaccines and serodiagnostics that are particularly effective for disease prevention and. . .

SUMM . . . of molecular biology. More specifically, the present invention relates to molecular cloning and characterization of homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , a multigene locus encoding the 28-kDa homologous proteins of ***Ehrlichia*** ***canis*** and uses thereof.

SUMM [0005] Canine ***ehrlichiosis*** , also known as canine tropical pancytopenia, is a tick-borne rickettsial disease of dogs first described in Africa in 1935 and. . .

SUMM [0006] The etiologic agent of canine ***ehrlichiosis*** is ***Ehrlichia*** ***canis*** , a small, gram-negative, obligate intracellular bacterium which exhibits tropism for mononuclear phagocytes (Nyindo et al., 1971) and is transmitted by the brown dog tick, Rhipicephalus sanguineus (Groves et al., 1975). The progression of canine ***ehrlichiosis*** occurs in three phases, acute, subclinical and chronic. The acute phase is characterized by fever, anorexia, depression, lymphadenopathy and mild. . .

SUMM . . . persistent infections in the host. Although disease pathogenesis is poorly understood, multigene families described in members of the related genera ***Ehrlichia*** , Anaplasma, and Cowdria may be involved in variation of major surface antigen expression thereby evading immune surveillance. Anaplasma marginale, an organism closely related to E. ***canis*** , exhibits variation of major surface protein 3 (msp-3) genes resulting in antigenic polymorphism among strains (Alleman et al., 1997).

SUMM [0008] Molecular taxonomic analysis based on the 16S rRNA gene has determined that E. ***canis*** and E. ***chaffeensis*** , the etiologic agent of human monocytic ***ehrlichiosis*** (HME), are closely related (Anderson et al., 1991; Anderson et al., 1992; Dawson et al., 1991; Chen et al., 1994). Considerable cross reactivity of the 64, 47, 40, 30, 29 and 23-kDa antigens between E. ***canis*** and E. ***chaffeensis*** has been reported (Chen et al., 1994; Chen et al., 1997; Rikihisa et al., 1994; Rikihisa et al., 1992). Analysis. . . with human and canine convalescent phase sera by immunoblot has resulted in the identification of numerous immunodominant proteins of E. ***canis*** , including a 30-kDa protein (Chen et al., 1997). In addition, a 30-kDa protein of E. ***canis*** has been described as a major immunodominant antigen recognized early in the immune response that is antigenically distinct from the 30-kDa protein of E. ***chaffeensis*** (Rikihisa et al., 1992; Rikihisa et al., 1994). Other immunodominant proteins of E. ***canis*** with molecular masses ranging from 20 to 30-kDa have also been identified (Brouqui et al., 1992; Nyindo et al., 1991;. . .

SUMM [0009] Homologous 28-32 kDa immunodominant proteins encoded by multigene

families have been reported in related organisms including, E. ***chaffeensis*** and Cowdria ruminantium (Sulsona et al., 1999; Ohashi et al., 1998a; Reddy et al., 1998). Recently, characterization of a 21 member multigene family encoding proteins of 23 to 28-kDa has been described in E. ***chaffeensis*** (Yu et al., 2000). The E. ***chaffeensis*** 28-kDa outer membrane proteins are surface exposed, and contain three major hypervariable regions (Ohashi et al., 1998a). The recombinant E. ***chaffeensis*** P28 appeared to provide protection against homologous challenge infection in mice, and antisera produced against the recombinant protein cross reacted with a 30-kDa protein of E. ***canis*** (Ohashi et al., 1998a). Diversity in the p28 gene among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a), and studies using monoclonal antibodies have further demonstrated diversity in the expressed P28 proteins (Yu et al., 1993). Conversely, complete conservation of a p28 genes in geographically different isolates of E. ***canis*** has been reported and suggests that E. ***canis*** may be conserved in North America (McBride et al., 1999, 2000).

SUMM . . . The prior art is deficient in the lack of cloning and characterization of new homologous 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** and a single multigene locus containing the homologous 28-kDa protein genes. Further, The prior art is deficient in the lack of recombinant proteins of such immunoreactive genes of ***Ehrlichia*** ***canis*** . The present invention fulfills this long-standing need and desire in the art.

SUMM . . . of the present invention describe the molecular cloning, sequencing, characterization, and expression of homologous mature 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** (designated p28-1, -2, -3, -5, -6, -7, -9), and the identification of a single locus (10,677-bp) containing nine 28-kDa protein genes of ***Ehrlichia*** ***canis*** (p28-1 to p28-9). Eight of the p28 genes were located on one DNA strand, and one p28 gene was found. . .

SUMM [0012] In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

SUMM . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

SUMM [0016] The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a

subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, e.g., the stimulation of the subject's humoral or cellular immune. . .

DRWD . . . arrow) and 16-kDa thioredoxin control (Lane 2, arrow), and corresponding immunoblot of recombinant p28-7-thioredoxin fusion protein recognized by covalescent-phase E. ***canis*** canine antiserum (Lane 3). Thiroredoxin control was not detected by E. ***canis*** antiserum (not shown).

DRWD . . . ID NO. 2), p28-5 protein (ECa28SA2, partial sequence, SEQ ID NO. 7), p28-4 protein (ECa28SA1, SEQ ID NO. 8), E. ***chaffeensis*** P28 (SEQ ID NO. 9), E. ***chaffeensis*** OMP-1 family (SEQ ID NOs: 10-14) and C. ruminantium MAP-1 protein (SEQ ID NO. 15). The p28-7 amino acid sequence. . .

DRWD [0022] FIG. 4 shows phylogenetic relatedness of E. ***canis*** p28-7 (ECa28-1), p28-5 (ECa28SA2, partial sequence), p28-4 (ECa28SA1), members of the E. ***chaffeensis*** omp-1 multiple gene family, and C. rumanintium map-1 protein from deduced amino acid sequences utilizing unbalanced tree construction. The length. . .

DRWD [0023] FIG. 5 shows Southern blot analysis of E. ***canis*** genomic DNA completely digested with six individual restriction enzymes and hybridized with a p28-7 DIG-labeled probe (Lanes 2-7); DIG-labeled molecular. . .

DRWD [0024] FIG. 6 shows comparison of predicted protein characteristics of E. ***canis*** p28-7 (ECa28-1, Jake strain) and E. ***chaffeensis*** P28 (Arkansas strain). Surface probability predicts the surface residues by using a window of hexapeptide. A surface residue is any. . .

DRWD [0025] FIG. 7 shows nucleic acid sequences and deduced amino acid sequences of the E. ***canis*** 28-kDa protein genes p28-5 (nucleotide 1-849: SEQ ID No. 3; amino acid sequence: SEQ ID No. 4) and p28-6 (nucleotide. . .

DRWD [0026] FIG. 8 shows schematic of the E. ***canis*** 28-kDa protein gene locus (5.592-Kb, containing five genes) indicating genomic orientation and intergenic noncoding regions (28NC1-4). The 28-kDa protein genes. . .

DRWD [0027] FIG. 9 shows phylogenetic relatedness of the E. ***canis*** 28-kDa protein gene p28-4 (ECa28SA1), p28-5 (ECa28SA2), p28-6 (ECa28SA3), p28-7 (ECa28-1) and p28-8 (ECa28-2) based on amino acid sequences utilizing. . .

DRWD [0028] FIG. 10 shows alignment of E. ***canis*** 28-kDa protein gene intergenic noncoding nucleic acid sequences (SEQ ID Nos. 30-33). Nucleic

acids not shown, denoted with a dot. . .

DRWD [0029] FIG. 11 shows schematic representation of the nine gene E. ***canis*** p28 locus (10,677-bp) indicating genomic orientation and intergenic noncoding regions. The p28 genes (p28-1, 2, 3, 9) (unshaded) were identified in Example 8. Shaded p28 genes have been identified previously and designated as follows: p28-4, ***p30a*** (Ohashi et al., 1998b) and ORFI (Reddy et al., 1998); p28-5 and p28-6, (McBride, et. al., 2000); p28-7, p28 (McBride et al., 1999) and ***p30*** (Ohashi et al., 1998b); and p28-8, ***p30*** -1 (Ohashi et al., 1998b).

DRWD [0030] FIG. 12 shows phylogenetic relationships of E. ***canis*** P28-1 to P28-9 based on the amino acid sequences. The length of each pair of branches represents the distance between. . .

DRWD . . . 13 shows nucleic acid sequence (SEQ ID No. 39) and deduced amino acid sequence (SEQ ID No. 40) of E. ***canis*** p28-1 gene.

DRWD . . . 14 shows nucleic acid sequence (SEQ ID No. 41) and deduced amino acid sequence (SEQ ID No. 42) of E. ***canis*** p28-2 gene.

DRWD . . . 15 shows nucleic acid sequence (SEQ ID No. 43) and deduced amino acid sequence (SEQ ID No. 44) of E. ***canis*** p28-3 gene.

DRWD . . . 16 shows nucleic acid sequence (SEQ ID No. 45) and deduced amino acid sequence (SEQ ID No. 46) of E. ***canis*** p28-9 gene.

DETD [0035] The present invention describes cloning, sequencing and expression of homologous genes encoding a 30-kilodalton (kDa) protein of ***Ehrlichia*** ***canis*** . A comparative molecular analysis of homologous genes among seven E. ***canis*** isolates and the E. ***chaffeensis*** omp-1 multigene family was also performed. Several new 28-kDa protein genes are identified as follows:

DETD [0038] Using PCR to amplify 28-kDa protein genes of E. ***canis*** , a previously unsequenced region of p28-5 (Eca28SA2) was completed. Sequence analysis of p28-5 revealed an 849-bp open reading frame encoding. . .

DETD . . . of tandemly arranged p28 genes were sequenced, and p28-1, -2, -3, and -9 were identified. Consequently, a nine gene E. ***canis*** p28 locus spanning 10, 677 bp was identified in the present invention.

DETD [0041] The present invention is directed to, inter alia, homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , p28-1, -2, -3, -6, -7, and p28-9, and a complete sequence of previously partially sequenced p28-5. Also disclosed is a multigene locus encoding nine homologous 28-kDa outer membrane proteins of ***Ehrlichia*** ***canis*** . Eight of the p28 genes were located on one DNA strand, and one p28 gene was found on the complementary. . .

DETD [0043] The invention includes a substantially pure DNA encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably. . .

DETD . . . listed in SEQ ID No 1, 3, 5, 39, 41, 43, or 45 which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** .

DETD . . . comprises a vector comprising a DNA sequence coding for a which encodes a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and said vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication;. . .

DETD . . . or viral nucleic acid. Vectors may be used to amplify and/or express nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to. . .

DETD . . . such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention can be used to transform a host using any of the techniques commonly known to those. . . Especially preferred is the use of a vector containing coding sequences for a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention for purposes of prokaryote transformation.

DETD . . . "recombinant" cell is intended to refer to a cell into which a recombinant gene, such as a gene encoding an ***Ehrlichia*** ***canis*** antigen has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced. . .

DETD [0053] The present invention is also drawn to substantially pure 28-30 kDa immunoreactive proteins of E. ***canis*** comprise of amino acid sequences listed in, for example, SEQ ID No. 2, 4, 6, 40, 42, 44, or 46.

DETD . . . more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** ; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography such as immunoaffinity chromatography using an antibody specific for a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from. . .

DETD . . . In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (SEQ ID No. 2, 4, 6, 40, 42, 44, or 46). As used herein, "fragment," as applied to a polypeptide,. . . 30 (e.g., 50) residues in length, but

less than the entire, intact sequence. Fragments of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , by recombinant DNA techniques using an expression vector that encodes a defined fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (e.g., binding to an antibody specific for 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis***) can be assessed by methods described herein.

DETD [0056] Purified 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** or antigenic fragments of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay). . .

DETD [0059] Included in this invention are polyclonal antisera generated by using 28-kDa immunoreactive protein of ***Ehrlichia*** can is or a fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant ***Ehrlichia*** ***canis*** cDNA clones, and to distinguish them from known cDNA clones.

DETD [0067] In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

DETD . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

DETD [0071] The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, i.e. the stimulation of the subject's humoral

or cellular immune. . .

DETD [0073] ***Ehrlichiae*** and Purification ***Ehrlichia*** ***canis*** (Florida strain and isolates Demon, D J, Jake, and Fuzzy) were provided by Dr. Edward Breitschwerdt, (College of Veterinary Medicine, North Carolina State University, Raleigh, N.C.). E. ***canis*** (Louisiana strain) was provided by Dr. Richard E. Corstvet (School of Veterinary Medicine, Louisiana State University, Baton Rouge, La.) and E. ***canis*** (Oklahoma strain) was provided by Dr. Jacqueline Dawson (Centers for Disease Control and Prevention, Atlanta, Ga.). Propagation of ***ehrlichiae*** was performed in DH82 cells with DMEM supplemented with 10% bovine calf serum and 2 mM L-glutamine at 37.degree. C. The intracellular growth in DH82 cells was monitored by presence of E. ***canis*** morulae using general cytologic staining methods. Cells were harvested when 100% of the cells were infected with ***ehrlichiae*** and were then pelleted in a centrifuge at 17,000.times.g for 20 min. Cell pellets were disrupted with a Braun-Sonic 2000 sonicator twice at 40W for 30 sec on ice. ***Ehrlichiae*** were purified as described previously (Weiss et al., 1975). The lysate was loaded onto discontinuous gradients of 42%-36%-30% renografin, and centrifuged at 80,000.times.g for 1 hr. Heavy and light bands containing ***ehrlichiae*** were collected and washed with sucrose-phosphate-glutamate buffer (SPG, 218 mM sucrose, 3.8 mM KH.sub.2PO.sub.4, 7.2 mM K.sub.2HPO.sub.4, 4.9 mM glutamate,. . .

DETD [0074] Nucleic Acid Preparation ***Ehrlichia*** ***canis*** genomic DNA was prepared by resuspending the renografin-purified ***ehrlichiae*** in 600 .mu.g of 10 mM Tris-HCl buffer (pH 7.5) with 1% sodium dodecyl sulfate (SDS, w/v) and 100 ng/ml. . .

DETD . . . determined using a Universal GenomeWalker Kit (CLONTECH, Palo Alto, Calif.) according to the protocol supplied by the manufacturer. Genomic E. ***canis*** (Jake isolate) DNA was digested completely with five restriction enzymes (DraI, EcoRV, PvuII, ScaI, StuI) which produce blunt-ended DNA. An adapter (AP1) supplied in the kit was ligated to each end of E. ***canis*** DNA. The genomic libraries were used as templates to find the unknown DNA sequence of the p28-7 gene by PCR. . .

DETD . . . with an ABI Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The entire p28-7 genes of seven E. ***canis*** isolates (four from North Carolina, and one each from Oklahoma, Florida, and Louisiana) were amplified by PCR with primers EC28OM-F. . .

DETD [0077] PCR Amplification, Cloning, Sequencing and Expression E. ***canis*** ECa28-1 (p28-7) Gene

DETD [0078] Expression Vectors The entire E. ***canis*** p28-7 gene was PCR-amplified with primers-EC28OM-F and EC28OM-R and cloned into pCR2.1-TOPO TA cloning vector to obtain the desired set. . .

DETD [0079] Western Blot Analysis Recombinant E. ***canis*** p28-7 fusion protein was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-15% Tris-HCl gradient gels (Bio-Rad, Hercules, Calif.) and transferred. . . . N.H.) using a semi-dry transfer cell (Bio-Rad, Hercules, Calif.). The membrane was incubated with convalescent phase antisera from an E. ***canis*** -infected dog diluted 1:5000 for 1 hour, washed, and then incubated with an anti-canine IgG (H & L) alkaline phosphatase-conjugated affinity-purified. . .

DETD [0080] Southern Blot Analysis To determine if multiple genes homologous to the p28-7 gene were present in the E. ***canis*** genome, a genomic Southern blot analysis was performed using a standard procedure (Sambrook et al. 1989). E. ***canis*** genomic DNA digested completely with each of the restriction enzymes BanII, EcoRV, HaeII, KpnI and SpeI, which do not cut. . . digested probe (566-bp) was separated by agarose gel electrophoresis, gel-purified and then used for hybridization. The completely digested genomic E. ***canis*** DNA was electrophoresed and transferred to a nylon membrane (Boehringer Mannheim, Indianapolis, Ind.) and hybridized at 40.degree. C. for 16. . .

DETD [0081] Sequence Analysis and Comparasion E. ***chaffeensis*** p28 and C. ruminantium map-1 DNA sequences were obtained from the National Center of Biotechnology Information (NCBI). Nucleotide and deduced. .

DETD [0082] Sequence analysis of p28-7 from seven different strains of E. ***canis*** was performed with primers designed to amplify the entire gene. Analysis revealed the sequence of this gene was conserved among. . .

DETD [0084] Alignment of nucleic acid sequences from E. ***chaffeensis*** p28 and Cowdria ruminantium map-1 using the Jotun-Hein aligorithm produced a consensus sequence with regions of high homology (>90%). These homologous regions (nucleotides 313-332 and 823-843 of C. ruminantium map-1; 307-326 and 814-834 of E. ***chaffeensis*** p28) were targeted as primer annealing sites for PCR amplification. PCR amplification of the E. ***canis*** p28-7 gene was accomplished with primers 793 (5-GCAGGAGCTGTTGGTTACTC-3') (SEQ ID NO. 16) and 1330 (5'-CCTTCCTCCAAGTTCTATGCC-3') (SEQ ID NO. 17), resulting in a 518-bp PCR product. E. ***canis*** DNA was amplified with primers 793 and 1330 with a thermal cycling profile of 95.degree. C. for 2 min, and. . . followed by a 72.degree. C. extension for 10 min and 4.degree. C. hold. The nucleic acid sequence of the E. ***canis*** PCR product was obtained by sequencing the product directly with primers 793 and 1330.

DETD . . . frame encoding a protein of 170 amino acids, and alignment of the 518-bp sequence obtained from PCR amplification of E. ***canis*** with the DNA sequence of E. ***chaffeensis*** p28 gene revealed a similarity greater than 70%, indicating that the genes were homologous.

DETD . . . PCR product amplified with these primers was sequenced directly with the same primers. The complete DNA sequence for the E. ***canis*** p28-7 gene (SEQ ID NO. 1) is shown in FIG. 1. The p28-7 PCR fragment amplified with these primers contained. . . amino acids from the multiple cloning site and 5' non-coding primer region at the N-terminus. Convalescent-phase antiserum from an E. ***canis*** infected dog recognized the expressed recombinant fusion protein, but did not react with the thioredoxin control (FIG. 2).

DETD [0089] Sequence Homology of E. ***canis*** p28-7 Gene

DETD [0090] The nucleic acid sequence of E. ***canis*** p28-7 (834-bp) and the E. ***chaffeensis*** omp-1 family of genes including signal sequences (p28-7, omp-1A, B, C, D, E, and F) were aligned using the Clustal method to examine homology between these genes (alignment not shown). Nucleic acid homology was equally conserved (68.9%) between E. ***canis*** p28-7, E. ***chaffeensis*** p28 and omp-1F. Other putative outer membrane protein genes in the E. ***chaffeensis*** omp-1 family, omp-1D (68.2%), omp-1E (66.7%), omp-1C (64.1%), Cowdria ruminantium map-1 (61.8%), E. ***canis*** 28-kDa protein 1 gene (60%) and 28-kDa protein 2 gene (partial) (59.5%) were also homologous to p28-7. E. ***chaffeensis*** omp-1B had the least nucleic acid homology (45.1%) with E. ***canis*** p28-7.

DETD [0091] Alignment of the predicted amino acid sequences of E. ***canis*** P28-7 (SEQ ID NO. 2) and E. ***chaffeensis*** P28 revealed amino acid substitutions resulting in four variable regions (VR). Substitutions or deletions in the amino acid sequence and the locations of variable regions of E. ***canis*** P28-7 and the E. ***chaffeensis*** OMP-1 family were identified (FIG. 3). Amino acid comparison including the signal peptide revealed that E. ***canis*** P28-7 shared the most homology with OMP-1F (68%) of the E. ***chaffeensis*** OMP-1 family, followed by E. ***chaffeensis*** P28 (65.5%), OMP-1E (65.1%), OMP-1D (62.9%), OMP-1C (62.9%), Cowdria ruminantium MAP-1 (59.4%), E. ***canis*** 28-kDa protein 1 (55.6%) and 28-kDa protein 2 (partial) (53.6%), and OMP-1B (43.2%). The phylogenetic relationships based on amino acid sequences show that E. ***canis*** P28-7 and C. ruminantium MAP-1, E. ***chaffeensis*** OMP-1 proteins, and E. ***canis*** 28-kDa proteins 1 and 2 (partial) are related (FIG. 4).

DETD [0092] Predicted Surface Probability and Immunoreactivity of E. ***canis*** P28-7

DETD [0093] Analysis of E. ***canis*** P28-7 using hydropathy and hydrophilicity profiles predicted surface-exposed regions on P28-7 (FIG. 6). Eight major surface-exposed regions consisting of 3 to 9 amino acids were identified on E. ***canis*** P28-7 and were similar to the profile of surface-exposed regions on E. ***chaffeensis*** P28 (FIG. 6). Five of the larger surface-exposed regions on E. ***canis***

P28-7 were located in the N-terminal region of the protein. Surface-exposed hydrophilic regions were found in all four of the variable regions of E. ***canis*** P28-7. Ten T-cell motifs were predicted in the P28-7 using the Rothbard-Taylor aligorithm (Rothbard and Taylor, 1988), and high antigenicity of the E. ***canis*** P28-7 was predicted by the Jameson-Wolf antigenicity aligorithm (FIG. 6) (Jameson and Wolf, 1988). Similarities in antigenicity and T-cell motifs were observed between E. ***canis*** P28-7 and E. ***chaffeensis*** P28.

DETD [0094] Detection of Homologous Genomic Copies of E. ***canis*** p28-7 Gene

DETD [0095] Genomic Southern blot analysis of E. ***canis*** DNA completely digested independently with restriction enzymes BanII, EcoRV, HaeII, KpnI, SpeI, which do not have restriction endonuclease sites in. . . . nucleotides 34, 43 and 656, revealed the presence of at least three homologous p28-7 gene copies (FIG. 5). Although E. ***canis*** p28-7 has internal Ase I internal restriction sites, the DIG-labeled probe used in the hybridization experiment targeted a region of. . .

DETD [0096] PCR Amplification of E. ***canis*** ECa28SA2 (p28-5), ECa28SA3 (p28-6) Genes and Identification of the Multiple Gene Locus

DETD [0101] Nucleic and Amino Acid Homology of E. ***canis*** p28-4, p28-5, p28-6, p28-7 and p28-8 Proteins

DETD [0102] The nucleic and amino acid sequences of all five E. ***canis*** 28-kDa protein genes were aligned using the Clustal method to examine the homology between these genes. The nucleic acid homology. . . 58 to 75% and a similar amino acid homology of ranging from 67 to 72% was observed between the E. ***canis*** 28-kDa protein gene members (FIG. 9).

DETD . . . protein genes were analyzed for promoter sequences by comparison with consensus Escherichia coli promoter regions and a promoter from E. ***chaffeensis*** (Yu et al., 1997; McClure, 1985). Putative promoter sequences including RBS, -10 and -35 regions were identified in 4 intergenic. . .

DETD [0104] N-Terminal Signal Sequence The amino acid sequence analysis revealed that entire E. ***canis*** p28-7 has a deduced molecular mass of 30.5-kDa and the entire p28-6 has a deduced molecular mass of 30.7-kDa. Both. . . N-terminal signal peptide of 23 amino acids (MNCKKILITTALMSLMYYAPSIS, SEQ ID No. 27), which is similar to that predicted for E. ***chaffeensis*** P28 (MNYKKILTSALISLISSLPGV SFS, SEQ ID NO. 28), and the OMP-1 protein family (Yu et al., 1999a; Ohashi et al., 1998b).

DETD . . . cleavage site at amino acid position 25 (MNCKKILITALISLMYSIPSISSFS, SEQ ID NO. 29) identical to the predicted cleavage site of E. ***chaffeensis*** P28 (SFS) was also present, and would result in a mature p28-7 with a predicted molecular mass of

27.7-kDa. Signal. . .

DETD [0106] Proteins of similar molecular mass have been identified and cloned from multiple rickettsial agents including E. ***canis*** , E. ***chaffeensis*** , and C. ruminantium (Reddy et al., 1998; Jongejan et al., 1993; Ohashi et al., 1998). A single locus in ***Ehrlichia*** ***chaffeensis*** with 6 homologous p28 genes, and 2 loci in E. ***canis*** , each containing some homologous 28-kDa protein genes have been previously described.

DETD [0107] The present invention demonstrated the cloning, expression and characterization of genes encoding mature 28-kDa proteins of E. ***canis*** that are homologous to the omp-1 multiple gene family of E. ***chaffeensis*** and the C. ruminantium map-1 gene. Two new 28-kDa protein genes were identified, p28-7 and p28-6. Another E. ***canis*** 28-kDa protein gene, p28-5, partially sequenced previously (Reddy et al., 1998), was sequenced completely in the present invention. Also disclosed is the identification and characterization of a single locus in E. ***canis*** containing five E. ***canis*** 28-kDa protein genes (p28-4, p28-5, p28-6, p28-7 and p28-8).

DETD [0108] The E. ***canis*** 28-kDa proteins are homologous to E. ***chaffeensis*** OMP-1 family and the MAP-1 protein of C. rumanintium. The most homologous E. ***canis*** 28-kDa proteins (p28-6, p28-7 and p28-8) are sequentially arranged in the locus. Homology of these proteins ranged from 67.5% to 72.3%. Divergence among these 28-kDa proteins was 27.3% to 38.6%. E. ***canis*** 28-kDa proteins p28-4 and p28-5 were the least homologous with homology ranging from 50.9% to 59.4% and divergence of 53.3. . . these regions are surface exposed and subject to selective pressure by the immune system. Conservation of p28-7 among seven E. ***canis*** isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be clonal in North America. Conversely, significant diversity of p28 among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a).

DETD [0109] All of the E. ***canis*** 28-kDa proteins appear to be post translationally processed from a 30-kD protein to a mature 28-kD protein. Recently, a signal sequence was identified on E. ***chaffeensis*** P28 (Yu et al., 1999a), and N-terminal amino acid sequencing has verified that the protein is post-translationally processed resulting in. . . OMP-1F and OMP-1E have also been proposed as leader signal peptides (Ohashi et al., 1998). Signal sequences identified on E. ***chaffeensis*** OMP-1F, OMP-1E and P28 are homologous to the leader sequence of E. ***canis*** 28-kDa protein. Promoter sequences for the p28 genes have not been determined experimentally, but putative promoter regions were identified by comparison with consensus sequences of the RBS, -10 and -35 promoter regions of E. coli and other ***ehrlichiae*** (Yu et al., 1997;

McClure, 1985). Such promoter sequences would allow each gene to potentially be transcribed and translated, suggesting. . .

DETD [0110] The E. ***canis*** 28-kda protein genes were found to exhibit nucleic acid and amino acid sequence homology with the E. ***chaffeensis*** omp-1 gene family and C. ruminantium map-1 gene. Previous studies have identified a 30-kDa protein of E. ***canis*** that reacts with convalescent phase antisera against E. ***chaffeensis*** , but was believed to be antigenically distinct (Rikihisa et al., 1994). Findings based on comparison of amino acid substitutions in four variable regions of E. ***canis*** 28-kDa proteins support this possibility. Together these findings also suggest that the amino acids responsible for the antigenic differences between E. ***canis*** and E. ***chaffeensis*** P28 are located in these variable regions and are readily accessible to the immune system.

DETD . . . It was reported that immunoreactive peptides were located in the variable regions of the 28-kDa proteins of C. ruminantium, E. ***chaffeensis*** and E. ***canis*** (Reddy et al., 1998). Analysis of E. ***canis*** and E. ***chaffeensis*** P28 revealed that all of the variable regions have predicted surface-exposed amino acids. A study in dogs demonstrated lack of cross protection between E. ***canis*** and E. ***chaffeensis*** (Dawson and Ewing, 1992). This observation may be related to antigenic differences in the variable regions of P28 as well as in other immunologically important antigens of these ***ehrlichial*** species. Another study found that convalescent phase human antisera from E. ***chaffeensis*** -infected patients recognized 29/28-kDa protein(s) of E. ***chaffeensis*** and also reacted with homologous proteins of E. ***canis*** (Chen et al., 1997). Homologous and crossreactive epitopes on the E. ***canis*** 28-kDa protein and E. ***chaffeensis*** P28 appear to be recognized by the immune system.

DETD [0112] E. ***canis*** 28-kDa proteins may be important immunoprotective antigens. Several reports have demonstrated that the 30-kDa antigen of E. ***canis*** exhibits strong immunoreactivity (Rikihisa et al., 1994; Rikihisa et al., 1992). Antibodies in convalescent phase antisera from humans and dogs have consistently reacted with proteins in this size range from E. ***chaffeensis*** and E. ***canis*** , suggesting that they may be important immunoprotective antigens (Rikihisa et al., 1994; Chen et al., 1994; Chen et al., 1997). In addition, antibodies to 30, 24 and 21-kDa proteins developed early in the immune response to E. ***canis*** (Rikihisa et al., 1994; Rikihisa et al., 1992), suggesting that these proteins may be especially important in the immune responses. . . Recently, a family of homologous genes encoding outer membrane proteins with molecular masses of 28-kDa have been identified in E. ***chaffeensis*** , and mice immunized with recombinant E.

chaffeensis P28 appeared to have developed immunity against homologous challenge (Ohashi et al., 1998). The P28 of E. ***chaffeensis*** has been demonstrated to be present in the outer membrane, and immunoelectron microscopy has localized the P28 on the surface. . . that it may serve as an adhesin (Ohashi et al., 1998). It is likely that the 28-kDa proteins of E. ***canis*** identified in this study have the same location and possibly serve a similar function.

DETD [0113] Comparison of p28-7 from different strains of E. ***canis*** revealed that the gene is apparently completely conserved. Studies involving E. ***chaffeensis*** have demonstrated immunologic and molecular evidence of diversity. Patients infected with E. ***chaffeensis*** have variable immunoreactivity to the 29/28-kDa proteins, suggesting that there is antigenic diversity (Chen et al., 1997). Recently molecular evidence has been generated to support antigenic diversity in the p28 gene from E. ***chaffeensis*** (Yu et al., 1999a). A comparison of five E. ***chaffeensis*** isolates revealed that two isolates (Sapulpa and St. Vincent) were 100% identical, but three others (Arkansas, Jax, 91HE17) were divergent by as much as 13.4% at the amino acid level. The conservation of E. ***canis*** p28-7 suggests that E. ***canis*** strains found in the United States may be genetically identical, and thus E. ***canis*** 28-kDa protein is an attractive vaccine candidate for canine ***ehrlichiosis*** in the United States. Further analysis of E. ***canis*** isolates outside the United States may provide information regarding the origin and evolution of E. ***canis*** . Conservation of the 28-kDa protein makes it an important potential candidate for reliable serodiagnosis of canine ***ehrlichiosis*** .

DETD [0114] The role of multiple homologous genes is not known at this point; however, persistence of E. ***canis*** infections in dogs could conceivably be related to antigenic variation due to variable expression of homologous 28-kDa protein genes, thus enabling E. ***canis*** to evade immune surveillance. Variation of msp-3 genes in A. marginale is partially responsible for variation in the MSP-3 protein, resulting in persistent infections (Alleman et al., 1997). Studies to examine 28-kDa protein gene expression by E. ***canis*** in acutely and chronically infected dogs would provide insight into the role of the 28-kDa protein gene family in persistence. . .

DETD [0115] Identification of E. ***canis*** p28-1, p28-2, p28-3 and p28-9 Genes

DETD [0117] The nucleic acid and amino acid sequences of the E. ***canis*** p28 genes were aligned using the Clustal method to examine the homology between these genes. Homology of these proteins ranged from 67.5% to 75%, and divergence among these P28 proteins was 26.9% to 38%. E. ***canis*** P28 proteins P28-1, P28-2, and P28-9 were the least

homologous with the other p28 genes ranging from 37% to 49%. . . nucleic acid homology of the nine p28 genes ranged from 28 to 72%. The phylogenetic relationships based on the E. ***canis*** p28 amino acid sequences are shown in FIG. 12.

DETD . . . accession numbers. The GenBank accession numbers for the nucleic acid and amino acid sequences for the complete nine gene E. ***canis*** (Jake strain) p28 gene locus is AF082744. This accession number was originally assigned to p28-7, but has been updated with. . . p28 locus, which includes p28-7. GenBank accession numbers for nucleic acid and amino acid sequences of p28-7 in other E. ***canis*** isolates described in this study are: Louisiana, AF082745; Oklahoma, AF082746; Demon, AF082747; DJ, AF082748; Fuzzy, AF082749; Florida, AF082750.

DETD [0119] Multiple bands in the 28-kilodalton range have been observed by immunoblots of convalescent sera from E. ***canis*** infected dogs (Rikihisa et al., 1994), and expression of multiple p28 proteins could be an explanation for this observation. Southern. . .

DETD [0120] In this study a single gene locus containing nine tandemly arranged E. ***canis*** p28 genes encoding homologous, but nonidentical, p28 genes was identified. The nine gene locus included four new p28 genes (FIGS.. . .

DETD [0121] The P28s of E. ***canis*** were found to be as closely related to 28-kilodalton proteins of other species such as E. ***chaffeensis*** as they are to themselves (McBride et al., 2000). Differences among the proteins are found primarily in several major hypervariable. . .

DETD [0122] Conservation of an E. ***canis*** p28 gene (p28-7) among seven geographically different isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be highly conserved in North America. Similarly, the 120-kDa glycoprotein of E. ***canis*** is also conserved among isolates in the United States (Yu et al., 1997). In contrast, both the 120-kDa and the 28-kDa protein genes of E. ***chaffeensis*** are divergent among isolates (Yu et al., 1999a; Chen et al., 1997). The diversity of the 28-kDa protein gene of E. ***chaffeensis*** appeared to result from point mutations in the hypervariable regions perhaps due to selective immune pressure (Yu et al., 1999a). These data suggest that E. ***canis*** may have been introduced into North America relatively recently, and this may account for the conservation that was observed among isolates. The conservation of p28 genes in E. ***canis*** isolates may provide an opportunity to develop vaccine and serodiagnostic antigens that are particularly effective for disease prevention and serodiagnosis.. . .

DETD [0152] Troy G. C., et al., (1990) Canine ***ehrlichiosis*** . In Infectious diseases of the dog and cat. Green C. E. (ed). Philidelphia: W. B. Sauders Co.

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 1
LENGTH: 1607
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-7
SEQUENCE: 1
attttattta ttaccaatct tatataatat attaaatttc tcttacaaaa atctctaatg 60
ttttatacct aatatatata ttctggcttg tatctacttt gcacttccac tattgttaat 120
ttatttcac tattttaggt gtaatatgaa ttgcaaaaaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 2
LENGTH: 278
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-7 protein
SEQUENCE: 2
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Ala Leu Ile Ser Leu
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 849
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: mat_peptide
OTHER INFORMATION: nucleic acid sequence of p28-5
SEQUENCE: 3
atgaattgta aaaaagtttt cacaataagt gcattgatat catccatata cttcctacct 60
aatgtctcat actctaaccc agtatatggt aacagtatgt atggtaattt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of p28-5 protein
SEQUENCE: 4
Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala Leu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 840
TYPE: DNA

ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: mat_peptide
OTHER INFORMATION: nucleic acid sequence of p28-6
SEQUENCE: 5
atgaattgca aaaaaattct tataacaact gcattaatgt cattaatgta ctatgctcca 60
agcatatctt tttctgatac tatacaagac gataacactg gtagcttcta. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of p28-6 protein
SEQUENCE: 6
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Ala Leu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7
LENGTH: 133
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: partial amino acid sequence of p28-5 protein
SEQUENCE: 7
Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 8
LENGTH: 287
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of p28-4 protien
SEQUENCE: 8
Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 9
LENGTH: 281
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** P28
SEQUENCE: 9
Met Asn Tyr Lys Lys Val Phe Ile Thr Ser Ala Leu Ile Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 10
LENGTH: 283
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1B
SEQUENCE: 10
Met Asn Tyr Lys Lys Ile Phe Val Ser Ser Ala Leu Ile Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 11
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1C
SEQUENCE: 11
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Ala Leu Pro
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 12
LENGTH: 286
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1D
SEQUENCE: 12
Met Asn Cys Glu Lys Phe Phe Ile Thr Thr Ala Leu Thr Leu Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 13
LENGTH: 278
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1E
SEQUENCE: 13
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Val Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 14
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:

OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1F
SEQUENCE: 14
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Thr Leu Val Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 19
LENGTH: 20
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
OTHER INFORMATION: primer used for sequencing 28-kDa protein genes in E. ***canis***
SEQUENCE: 19
agtgcagagt cttcggtttc 20
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 20
LENGTH: 18
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
OTHER INFORMATION: primer used for sequencing 28-kDa protein genes in E. ***canis***
SEQUENCE: 20
gttacttgcg gaggacat 18
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 21
LENGTH: 24
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
LOCATION: nucleotides 687-710 of E. ***canis*** p28-7
OTHER INFORMATION: primer 394 for PCR
SEQUENCE: 21
gcatttccac aggatcatag gtaa 24
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 22
LENGTH: 24
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
LOCATION: nucleotides 710-687 of E. ***canis*** p28-7

OTHER INFORMATION: primer 394C for PCR
SEQUENCE: 22
ttacctatga tcctgtggaa atgc 24
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 23
LENGTH: 20
TYPE: DNA
ORGANISM: artificial sequence
FEATURE:
NAME/KEY: primer_bind
OTHER INFORMATION: primer 793C which anneals to a region with E. ***canis*** p28-7, used to amplify the intergenic region between gene p28-6 and p28-7
SEQUENCE: 23
gagtaaccaa cagctcctgc 20
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 27
LENGTH: 23
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
NAME/KEY: PEPTIDE
OTHER INFORMATION: a predicted N-terminal signal peptide of p28-7 and p28-6
SEQUENCE: 27
Met Asn Cys Lys Lys Ile Leu Ile. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 28
LENGTH: 25
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
FEATURE:
OTHER INFORMATION: amino acid sequence of N-terminal signal peptide of E. ***chaffeensis*** P28
SEQUENCE: 28
Met Asn Tyr Lys Lys Ile Leu Ile Thr Ser Ala Leu Ile Ser Leu
5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 29
LENGTH: 26
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of putative cleavage site of p28-7
SEQUENCE: 29
Met Asn Cys Lys Lys Ile Leu Ile Thr. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 30
LENGTH: 299
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 1 (28NC1)
SEQUENCE: 30
taatacttct attgtacatg ttaaaaatag tactagtttg cttctgtggt ttataaacgc 60
aagagagaaa tagttagtaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 31
LENGTH: 345
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 2 (28NC2)
SEQUENCE: 31
taatttcgtg gtacacatat cacgaagcta aaattgtttt tttatctctg ctgtatacaa 60
gagaaaaaat agtagtgaaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 32
LENGTH: 345
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 3 (28NC3)
SEQUENCE: 32
tgattttatt gttgccacat attaaaaatg atctaaactt gtttttatta ttgctacata 60
caaaaaaaag aaaaatagtg. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 33
LENGTH: 355
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 4 (28NC4)
SEQUENCE: 33
taattttatt gttgccacat attaaaaatg atctaaactt gtttttawta ttgctacata 60
caaaaaaaga aaaatagtgg. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 39
LENGTH: 879
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-1
SEQUENCE: 39
atgaataata aactcaaatt tactataata aacacagtat tagtatgctt attgtcatta 60
cctaatatat cttcctcaaa ggccataaac aataacgcta aaaagtacta cggattatat 120
atcagtggac aatataaacc cagtgtttct gttttcagta. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 40
LENGTH: 293
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-1 protein
SEQUENCE: 40
Met Asn Asn Lys Leu Lys Phe Thr Ile Ile Asn Thr Val Leu Val
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 41
LENGTH: 840
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-2
SEQUENCE: 41
atgaattata agaaaattct agtaagaagc gcgttaatct cattaatgtc aatcttacca 60
tatcagtctt ttgcagatcc tgtaggttca agaactaatg atacacaaga aggcttctac 120
attagtgcaa agtacaatcc aagtatatca cactttagaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 42
LENGTH: 280
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-2 protein
SEQUENCE: 42
Met Asn Tyr Lys Lys Ile Leu Val Arg Ser Ala Leu Ile Ser Leu
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 43
LENGTH: 828
TYPE: DNA

ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-3
SEQUENCE: 43
atgaactgta aaaaaattct tataacaact acattggtat cactaacaat tcttttacct 60
ggcatatctt tctccaaacc aatacatgaa aacaatacta caggaaactt ttacattatt 120
ggaaaatatg taccaagtat ttcacatttt gggaactttt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 44
LENGTH: 276
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-3 protein
SEQUENCE: 44
Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Thr Leu Val Ser Leu
5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 45
LENGTH: 813
TYPE: DNA
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-9
SEQUENCE: 45
atgaattaca aaagatttgt tgtaggtgtt acgctgagta catttgtttt tttcttatct 60
gatggtgctt tttctgatgc aaatttttct gaagggagga gaggacttta tataggtagt 120
cagtataaag ttggtattcc caattttagt aatttttcag. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 46
LENGTH: 271
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
FEATURE:
OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-9 protein
SEQUENCE: 46
Met Asn Tyr Lys Arg Phe Val Val Gly Val Thr Leu Ser Thr Phe
5. . .
CLM What is claimed is:

1. An isolated DNA sequence encoding a 30-kilodalton protein of ***Ehrlichia*** ***canis*** , wherein said protein is immunoreactive with anti- ***Ehrlichia*** ***canis*** serum.

6. The DNA sequence of claim 1, wherein said DNA is contained in a single locus of ***Ehrlichia*** ***canis*** .

8. The DNA sequence of claim 7, wherein said locus contains genes encoding homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** .

9. The DNA sequence of claim 8, wherein said homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** are selected from the group consisting of p28-1, p28-2, p28-3, p28-4, p28-5, p28-6, p28-7, p28-8 and p28-9.

17. A method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit ***Ehrlichia*** ***canis*** infection.

L7 ANSWER 6 OF 30 USPATFULL
AN 2003:86817 USPATFULL
TI Immune modulation method using steroid compounds
IN Ahlem, Clarence N., San Diego, CA, UNITED STATES
Frincke, James M., San Diego, CA, UNITED STATES
dos Anjos de Carvalho, Luis Daniel, Paio Pires, PORTUGAL
Heggie, William, Palmela, PORTUGAL
Prendergast, Patrick T., County Kildare, IRELAND
Reading, Christopher L., San Diego, CA, UNITED STATES
Thadikonda, Krupakar Paul, Gaithersburg, MD, UNITED STATES
Vernon, Russell N., Oak Hills, CA, UNITED STATES
PI US 2003060425 A1 20030327
AI US 2001-820483 A1 20010329 (9)
RLI Continuation-in-part of Ser. No. US 1999-449184, filed on 24 Nov 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-414905, filed on 8 Oct 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-449004, filed on 24 Nov 1999, ABANDONED Continuation-in-part of Ser. No. US 2000-535675, filed on 23 Mar 2000, PENDING Continuation-in-part of Ser. No. US 1999-449042, filed on 24 Nov 1999, ABANDONED Continuation-in-part of Ser. No. US 2000-675470, filed on 28 Sep 2000, PENDING Continuation-in-part of Ser. No. US 2000-586673, filed on 1 Jun 2000, ABANDONED Continuation-in-part of Ser. No. US 2000-586672, filed on 1 Jun 2000, ABANDONED Continuation-in-part of Ser. No. US 1999-461026, filed on 15 Dec 1999, ABANDONED
PRAI US 1998-109924P 19981124 (60)
US 1999-140028P 19990616 (60)
US 1998-109923P 19981124 (60)

US 1999-126056P 19991019 (60)
US 1999-124087P 19990311 (60)
US 1998-110127P 19981127 (60)
US 1999-161453P 19991025 (60)
US 1999-145823P 19990727 (60)
US 1999-137745P 19990603 (60)
US 1998-112206P 19981215 (60)
US 2000-257071P 20001220 (60)
DT Utility
FS APPLICATION
LREP HOLLIS-EDEN PHARMACEUTICALS, INC., 4435 EASTGATE MALL, SUITE 400, SAN
DIEGO, CA, 92121
CLMN Number of Claims: 54
ECL Exemplary Claim: 1
DRWN 6 Drawing Page(s)
LN.CNT 14708
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions comprising formula 1 steroids, e.g., 16.alpha.-bromo-3 .beta.-hydroxy-5.alpha.-androstan-17-one hemihydrate and one or more excipients, including compositions that comprise a liquid formulation comprising less than about 3% v/v water. The compositions are useful to make improved pharmaceutical formulations. The invention also provides methods of intermittent dosing of steroid compounds such as analogs of 16.alpha.-bromo-3.beta.-hydroxy-5.alpha.-androstan-17-one and compositions useful in such dosing regimens. The invention further provides compositions and methods to inhibit pathogen replication, ameliorate symptoms associated with immune dysregulation and to modulate immune responses in a subject using the compounds. The invention also provides methods to make and use these immunomodulatory compositions and formulations.
DETD . . . (e.g., L. donovani, L. major, L. braziliensis), Plasmodium sp. (e.g., P. falciparum, P. knowlesi, P. vivax, P. berghei, P. yoelli), ***Ehrlichia*** sp. (e.g., E. ***canis*** , E. ***chaffeensis*** , E. phagocytophila, E. equi, E. sennetsu), Entamoeba sp., Babesia microti, Haemophilus sp. (e.g., H. somnus, H. influenzae), Brucella sp. (e.g.,. . .
DETD . . . Osf2, Cbfa1, RUNX2, steroid receptor coactivator-1 family (SRC-1, SRC-1/serum response factor), SET, nerve growth factor inducible protein B, StF-IT, NFAT, ***p300*** , CREB, CREB-binding protein (CPB), ***p300*** /CBP, ***p300*** /CPB-associated factor, SWI/SNF and their human and other homologs, BRG-1, OCT-1/OAF, AP1, Ets, androgen receptor associated protein 54 (ARA54), androgen receptor. . . acute regulatory protein gene promoter region, RevErb, Rev-erbA .alpha., Rev-erb .beta., steroid receptor coactivator amplified in breast cancer

(AIB 1), ***p300*** /CREB binding protein-interacting protein (p/CIP), thyroid hormone receptor (TR, T3R), thyroid hormone response elements (T3REs), constitutive androstane receptor (CAR), Xenopus xSRC-3. . . receptor (TR3), RLD-1, OR-1, androgen receptor, glucocorticoid receptor, estrogen receptor, progesterone receptor, mineralcorticoid receptor, aldosterone receptor, OR1, OR1/RXR.alpha. complex, TIF-1, CBP/ ***P300*** complex, TRIP1/SUG-1 complex, RIP-140, steroid receptor coactivator 1 (SRC1), SRC1.alpha./P160 complex and TIF-2/GRIP-1 complex, RAR/N--CoR/RIP13 complex, RAR/SMRT/TRAC-2 complex, and the. . .

DETD . . . Exemplary transcription factors that may be present include one or more of ARA54, ARA55, ARA70, SRC-1, NF-.kappa.B, NFAT, AP1, Ets, ***p300*** , CBP, ***p300*** /CBP, ***p300*** /CPB-associated factor, SWI/SNF and human homologs of SWI/SNF, CBP, SF-1, RIP140, GRIP1 and Vpr. In general, one provides a first and. . .

DETD . . . cell or tissue extract or the partially purified or purified cell or tissue extract. The transactivator protein may be TIF-1, CBP/ ***P300*** , TRIP1/SUG-1, RIP-140, SRC1.alpha./P160, or TIF-2/GRIP-1. In any of these embodiments the complex comprising the steroid receptor protein, the formula 1. . .

L7 ANSWER 7 OF 30 USPATFULL

AN 2003:71974 USPATFULL

TI Immunostimulatory nucleic acid molecules

IN Krieg, Arthur M., Iowa City, IA, UNITED STATES
Kline, Joel, Iowa City, IA, UNITED STATES
Klinman, Dennis, Potomac, MD, UNITED STATES
Steinberg, Alfred D., Potomac, MD, UNITED STATES

PI US 2003050261 A1 20030313

AI US 2001-818918 A1 20010327 (9)

RLI Division of Ser. No. US 1996-738652, filed on 30 Oct 1996, GRANTED, Pat. No. US 6207646 Continuation-in-part of Ser. No. US 1995-386063, filed on 7 Feb 1995, GRANTED, Pat. No. US 6194388 Continuation-in-part of Ser. No. US 1994-276358, filed on 15 Jul 1994, ABANDONED

DT Utility

FS APPLICATION

LREP WOLF GREENFIELD & SACKS, PC, FEDERAL RESERVE PLAZA, 600 ATLANTIC AVENUE,
BOSTON, MA, 02210-2211

CLMN Number of Claims: 18

ECL Exemplary Claim: 1

DRWN 19 Drawing Page(s)

LN.CNT 2569

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Nucleic acids containing unmethylated CpG dinucleotides and therapeutic

utilities based on their ability to stimulate an immune response and to redirect a Th2 response to a Th1 response in a subject are disclosed. Methods for treating atopic diseases, including atopic dermatitis, are disclosed.

SUMM . . . interferon B gene". Proc. Natl. Acad. Sci. USA 89:2150, 1992), TGF-.beta.1 (Asiedu, C. K., L. Scott, R. K. Assoian, M. ***Ehrlich*** : "Binding of AP-1/CREB proteins and of MDBP to contiguous sites downstream of the human TGF-B1 gene". Biochim. Biophys. Acta 1219:55,. . .

SUMM . . . that E1A binds to the CREB-binding protein, CBP (Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner: "E1A-associated ***p300*** and CREB-associated CBP belong to a conserved family of coactivators". Cell 77:799, 1994). Human T lymphotropic virus-I (HTLV-1), the retrovirus. . .

DETD . . . spores and drugs (e.g. penicillin). Examples of natural, animal and plant allergens include proteins specific to the following genera: Canine (***Canis*** familiaris); Dermatophagoides (e.g. Dermatophagoides farinae); Felis (Felis domesticus); Ambrosia (Ambrosia artemiisfolia; Lolium (e.g. Lolium perenne or Lolium multiflorum); Cryptomeria (Cryptomeria. . .

L7 ANSWER 8 OF 30 CABA COPYRIGHT 2003 CABI DUPLICATE 2

AN 2003:76257 CABA

DN 20033045715

TI Transcriptional analysis of ***p30*** major outer membrane protein genes of ***Ehrlichia*** ***canis*** in naturally infected ticks and sequence analysis of ***p30*** -10 of E. ***canis*** from diverse geographic regions

AU Felek, S.; Greene, R.; Rikihisa, Y.

CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH 43210-1093, USA.

SO Journal of Clinical Microbiology, (2003) Vol. 41, No. 2, pp. 886-888. 18 ref.
Publisher: American Society for Microbiology (ASM). Washington
ISSN: 0095-1137

CY United States

DT Journal

LA English

AB Rhipicephalus sanguineus ticks transmit ***Ehrlichia*** ***canis*** , the etiologic agent of canine ***ehrlichiosis*** . In experimentally infected ticks, only ***p30*** -10 transcript was detected among 22 ***p30*** paralogs encoding immunodominant major outer membrane ***P30*** proteins of E. ***canis*** . The present study revealed transcription of ***p30*** -10 by E. ***canis*** in naturally infected ticks and sequence conservation of ***p30*** -10 genes for E.

canis from diverse geographic regions.

TI Transcriptional analysis of ***p30*** major outer membrane protein genes of ***Ehrlichia*** ***canis*** in naturally infected ticks and sequence analysis of ***p30*** -10 of E. ***canis*** from diverse geographic regions.

AB Rhipicephalus sanguineus ticks transmit ***Ehrlichia*** ***canis*** , the etiologic agent of canine ***ehrlichiosis*** . In experimentally infected ticks, only ***p30*** -10 transcript was detected among 22 ***p30*** paralogs encoding immunodominant major outer membrane ***P30*** proteins of E. ***canis*** . The present study revealed transcription of ***p30*** -10 by E. ***canis*** in naturally infected ticks and sequence conservation of ***p30*** -10 genes for E. ***canis*** from diverse geographic regions.

BT ***Ehrlichia*** ; ***Ehrlichiaceae*** ; Rickettsiales; bacteria; prokaryotes; Rhipicephalus; Ixodidae; Metastigmata; Acari; Arachnida; arthropods; invertebrates; animals

ORGN ***Ehrlichia*** ***canis*** ; Rhipicephalus sanguineus

L7 ANSWER 9 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3

AN 2002:501879 BIOSIS

DN PREV200200501879

TI Methods for detecting ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** in vertebrate and invertebrate hosts.

AU Stich, Roger William (1); Rikihisa, Yasuko

CS (1) Columbus, OH USA
ASSIGNEE: The Ohio State University Research Foundation

PI US 6432649 August 13, 2002

SO Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 13, 2002) Vol. 1261, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.

DT Patent

LA English

AB Tools and methods for detecting the presence of E. ***canis*** and E. ***chaffeensis*** in a sample obtained from an animal are provided. The methods employ a polymerase chain reaction and primer sets that are based on the ***p30*** gene of E. ***canis*** and the p28 gene of E. ***chaffeensis*** . The present invention also relates to the ***p30*** and the p28 primer sets. Each ***p30*** primer set comprises a first primer and the second primer, both of which are from 15 to 35 nucleotides in length. The first ***p30*** primer comprises a sequence which is complementary to a consecutive sequence, within the following sequence:

CCA AGTGTCTCAC ATTTTGGTAG CTTCTCAGCT AAAGAAGAAA GCAAATCAAC
TGTTGGAGTTTTTGGATTAA AACATGATTG GGATGGAAGT CCAATACTTA
AGAATAAACA
CGCTGACTTTACTGTTCCAA AC. SEQ ID NO.1. The second ***p30*** primer
comprises a sequence which is complementary to the inverse complement of a
consecutive sequence contained within the following sequence: GTTACT
CAATGGGTGG CCCAAGAATA GAATTCGAAA TATCTTATGA AGCATTCGAC
GTAAAAAGTC
CTAATATCAA TTATCAAAAT GACGCGCACA GGTACTGCGC TCTATCTCAT
CACACATCGG CAGCCAT,
SEQ ID NO.2. The first p28 comprises a sequence which is complementary to
a consecutive sequenc, within the following sequence : A GTTTTCATAA
CAAGTGCATT GATATCACTA ATATCTTCTC TACCTGGAGT ATCATTTTCC
GACCCAACAG
GTAGTGGTAT TAACGG, SEQ ID NO. 3. The second p28 primer comprises a
sequence which is complementary to the inverse complement of a consecutive
sequence within one of the following two sequences: CAT TTCTAGGTTT
TGCAGGAGCT ATTGGCTACT CAATGGATGG TCCAAGAATA GAGCTTGAAG
TATCTTATGA, SEQ ID
NO. 4, or C AAGGAAAGTT AGGTTTAAGC TACTCTATAA GCCCAGA, SEQ ID NO. 5.

TI Methods for detecting ***Ehrlichia*** ***canis*** and
Ehrlichia ***chaffeensis*** in vertebrate and invertebrate
hosts.

AB Tools and methods for detecting the presence of E. ***canis*** and E.
chaffeensis in a sample obtained from an animal are provided. The
methods employ a polymerase chain reaction and primer sets that are based
on the ***p30*** gene of E. ***canis*** and the p28 gene of E.
chaffeensis . The present invention also relates to the ***p30***
and the p28 primer sets. Each ***p30*** primer set comprises a first
primer and the second primer, both of which are from 15 to 35 nucleotides
in length. The first ***p30*** primer comprises a sequence which is
complementary to a consecutive sequence, within the following sequence:
CCA AGTGTCTCAC ATTTTGGTAG CTTCTCAGCT AAAGAAGAAA GCAAATCAAC
TGTTGGAGTTTTTGGATTAA AACATGATTG GGATGGAAGT CCAATACTTA
AGAATAAACA
CGCTGACTTTACTGTTCCAA AC. SEQ ID NO.1. The second ***p30*** primer
comprises a sequence which is complementary to the inverse complement of a
consecutive sequence contained within the following sequence:. . .

ORGN Super Taxa
Invertebrata: Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and
Chlamydias, Eubacteria, Bacteria, Microorganisms; Vertebrata: Chordata,
Animalia

ORGN Organism Name
Ehrlichia ***canis*** (Rickettsiaceae): pathogen;
Ehrlichia ***chaffeensis*** (Rickettsiaceae): pathogen;

invertebrate (Invertebrata): host; vertebrate (Vertebrata): host
ORGN Organism Superterms
Animals; Bacteria; Chordates; Eubacteria; Invertebrates; Microorganisms; Nonhuman Vertebrates; Vertebrates
GEN ***Ehrlichia*** ***canis*** ***p30*** gene (Rickettsiaceae); ***Ehrlichia*** ***chaffeensis*** p28 gene (Rickettsiaceae)

L7 ANSWER 10 OF 30 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
AN 2002:658778 CAPLUS
DN 137:212016
TI Outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochemical methods for diagnosing infections
IN Rikihisa, Yasuko; Ohashi, Norio
PA USA
SO U.S. Pat. Appl. Publ., 49 pp., Division of U.S. Ser. No. 314,701.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002120115	A1	20020829	US 2002-59964	20020128
US 6544517	B1	20030408	US 1999-314701	19990519
US 20030103991	US	20030605	US 2002-314639	20021209

PRAI US 1999-314701 A3 19990519
US 1998-100843P P 19980918
AB Diagnostic tools for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans, are provided. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the " ***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The ***P30F*** proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3; ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. ***chaffeensis*** OMP proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** protein are also provided. The present invention also relates to kits contg. reagents for diagnosing

human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compns. contg. one or more OMP proteins or ***P30F*** proteins.

TI Outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochemical methods for diagnosing infections

AB Diagnostic tools for serodiagnosing ***ehrlichiosis*** in mammals, particularly in members of the Canidae family and in humans, are provided. The diagnostic tools are a group of outer membrane proteins of E. ***chaffeensis*** and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of E. ***canis*** and variants thereof referred to hereinafter as the " ***P30F*** proteins", and antibodies to the OMP proteins and the ***P30F*** proteins. The OMP proteins of E. ***chaffeensis*** encompass OMP-1, OMP-1A, OMP1-B, OMP-1C, OMP1-D, OMP1-E, OMP1-F, OMP1-H, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z. The ***P30F*** proteins of E. ***canis*** encompass ***P30*** , ***P30a*** , ***P30*** -1, ***P30*** -2, ***P30*** -3; ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, ***P30*** -10, ***P30*** -11, and ***P30*** -12. Isolated polynucleotides that encode the E. ***chaffeensis*** OMP proteins and isolated polynucleotides that encode the E. ***canis*** ***P30F*** protein are also provided. The present invention also relates to kits contg. reagents for diagnosing human ***ehrlichiosis*** and canine ***ehrlichiosis*** , and to immunogenic compns. contg. one or more OMP proteins or ***P30F*** proteins.

ST sequence ***Ehrlichia*** OMP protein gene; human canine ***ehrlichiosis*** diagnosis immunoassay

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1A; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1H; outer membrane proteins and

genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1R; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1S; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1T; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1U; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1V; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1W; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1X; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins

RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1Y; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), OMP-1Z; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -10; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -11; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -12; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -1; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -2; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -3; outer membrane proteins

and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -4; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -5; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -6; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -7; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -8; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** -9; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30*** ; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins

RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein), ***P30a*** ; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Proteins
RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(OMP (outer membrane protein); outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Antibodies
RL: ARG (Analytical reagent use); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(anti-OMP; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Immunoassay
(enzyme, dot-blot; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Immunoassay
(immunoblotting; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Diagnosis
(mol.; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT DNA sequences
(of outer membrane protein genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Protein sequences
(of outer membrane proteins of ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Dog (***Canis*** familiaris)
Ehrlichia ***canis***
Ehrlichia ***chaffeensis***
Human
Immunoassay
Test kits
(outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT 454740-64-8 454740-65-9 454740-66-0 454740-67-1 454740-68-2
454740-69-3 454740-70-6 454740-71-7 454740-72-8 454740-73-9
454740-74-0 454740-75-1 454740-76-2 454740-77-3 454740-78-4
454740-79-5 454740-80-8 454740-81-9 454740-82-0 454740-83-1
454740-84-2 454740-85-3 454740-86-4 454740-87-5 454740-88-6
454740-89-7 454740-90-0 454740-91-1 454740-92-2 454740-93-3
454740-94-4 454740-95-5 454740-96-6 454740-97-7 454740-98-8
454740-99-9 454741-00-5 454741-01-6 454741-02-7 454741-03-8
454741-04-9 454741-05-0 454741-06-1 454741-07-2 454741-08-3
454741-09-4 454741-10-7 454741-11-8
RL: ARG (Analytical reagent use); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(amino acid sequence; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT 454740-16-0 454740-17-1 454740-18-2 454740-19-3 454740-20-6
454740-21-7 454740-22-8 454740-23-9 454740-24-0 454740-25-1
454740-26-2 454740-27-3 454740-28-4 454740-29-5 454740-30-8
454740-31-9 454740-32-0 454740-33-1 454740-34-2 454740-35-3
454740-36-4 454740-37-5 454740-38-6 454740-39-7 454740-40-0
454740-41-1 454740-42-2 454740-43-3 454740-44-4 454740-45-5
454740-46-6 454740-47-7 454740-48-8 454740-49-9 454740-50-2
454740-51-3 454740-52-4 454740-53-5 454740-54-6 454740-55-7
454740-56-8 454740-57-9 454740-58-0 454740-59-1 454740-60-4
454740-61-5 454740-62-6 454740-63-7
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT 392038-87-8, GenBank U72291 392038-88-9, GenBank AF021338
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT 454741-84-5 454741-86-7 454741-88-9 454741-90-3 454741-92-5
454741-94-7 454741-96-9 454741-97-0 454741-98-1
RL: PRP (Properties)
(unclaimed nucleotide sequence; outer membrane proteins and genes of

Ehrlichia ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT 454741-85-6 454741-87-8 454741-89-0 454741-91-4 454741-93-6 454741-95-8
RL: PRP (Properties)
(unclaimed protein sequence; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

IT 454688-26-7 454741-99-2
RL: PRP (Properties)
(unclaimed sequence; outer membrane proteins and genes of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and immunochem. methods for diagnosing infections)

L7 ANSWER 11 OF 30 CAPLUS COPYRIGHT 2003 ACS
AN 2002:555769 CAPLUS
DN 137:124190
TI ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***
IN Lawton, Robert; O'Connor, Thomas Patrick, Jr.; Bartol, Barbara Ann; Machenry, Paul Scott
PA Idexx Laboratories, Inc., USA
SO PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002057794	A2	20020725	WO 2002-US1395	20020116
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002177178	A1	20021128	US 2001-765739	20010118
US 2002160432	A1	20021031	US 2002-54647	20020122
PRAI US 2001-765739	A	20010118		

AB The invention provides methods and compns. for the detection of

Ehrlichia ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies and antibody fragments. The antigenic epitopes are identified using phage display technol. and are derived from ***Ehrlichia*** ***canis*** ***P30*** -1, ***P30*** , P28, OMP-1C, OMP-1D, OMP-1E, and OMP-1F. These antigenic epitope polypeptides are used in reversible flow chromatog. binding assay, ELISA, western blot, or indirect FIA for detecting the presence of antibodies or fragments to ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** .

TI ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***

AB The invention provides methods and compns. for the detection of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies and antibody fragments. The antigenic epitopes are identified using phage display technol. and are derived from ***Ehrlichia*** ***canis*** ***P30*** -1, ***P30*** , P28, OMP-1C, OMP-1D, OMP-1E, and OMP-1F. These antigenic epitope polypeptides are used in reversible flow chromatog. binding assay, ELISA, western blot, or indirect FIA for detecting the presence of antibodies or fragments to ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** .

ST immunodiagnosis antigen epitope antibody ***Ehrlichia*** infection

IT Carriers
Chromatographic stationary phases
Chromatographs
Dog (***Canis*** familiaris)
Ehrlichia
Ehrlichia ***canis***
Ehrlichia ***chaffeensis***
Epitopes
Human
Indicators
Mammalia
Protein sequences
(***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Antibodies
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Proteins

RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(OMP (outer membrane protein), OMP-1C; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Proteins
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(OMP (outer membrane protein), OMP-1D; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Proteins
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(OMP (outer membrane protein), OMP-1E; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Proteins
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(OMP (outer membrane protein), OMP-1F; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Antigens
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(***P30*** -1; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Antigens
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(***P30*** ; ***Ehrlichia*** antigenic peptides for diagnosis of

infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Immunoassay
(app.; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Samples
(biol.; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Immunoassay
(enzyme-linked immunosorbent assay; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Immunoassay
(fluorescence, indirect; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Immunoglobulins
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(fragments; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Reversed phase chromatography
(immunoassay; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Immunoassay
(immunoblotting; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Diagnosis
(immunodiagnosis; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Reagents
RL: ARG (Analytical reagent use); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(immunodiagnostic; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Antibodies
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); BIOL (Biological study); USES (Uses)

(monoclonal; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Proteins

RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)

(p28; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT Immunoassay

(reverse flow chromatog.; ***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

IT 444069-23-2 444069-24-3 444069-25-4 444069-26-5 444069-27-6 444069-28-7 444069-29-8

RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DEV (Device component use); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(***Ehrlichia*** antigenic peptides for diagnosis of infections by ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis***)

L7 ANSWER 12 OF 30 USPATFULL

AN 2002:314701 USPATFULL

TI Compositions and methods for detection of ***ehrlichia*** ***canis*** and ***ehrlichia*** ***chaffeensis*** antibodies

IN Lawton, Robert, Gorham, ME, UNITED STATES
O'Connor, Thomas Patrick, JR., Westbrook, ME, UNITED STATES
Bartol, Barbara Ann, Gorham, ME, UNITED STATES
MacHenry, Paul Scott, Portland, ME, UNITED STATES

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DT Utility

FS APPLICATION

LREP MCDONNELL BOEHNEN HULBERT & BERGHOFF, 300 SOUTH WACKER DRIVE, SUITE 3200, CHICAGO, IL, 60606

CLMN Number of Claims: 34

ECL Exemplary Claim: 1

DRWN No Drawings

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods and compositions for the detection of

Ehrlichia ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies and antibody fragments.

TI Compositions and methods for detection of ***ehrlichia*** ***canis*** and ***ehrlichia*** ***chaffeensis*** antibodies

AB The invention provides methods and compositions for the detection of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies and antibody fragments.

SUMM [0001] The invention provides compositions and methods for the detection and quantification of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies and antibody fragments.

SUMM [0002] The Ehrilichia are obligate intracellular pathogens that infect circulating lymphocytes in mammalian hosts. ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** are members of the same sub-genus group that infect canines and humans and cause canine monocytic ***ehrlichiosis*** (CME) and human monocytic ***ehrlichiosis*** (HME), respectively. The canine disease is characterized by fever, lymphadenopathy, weight loss, and pancytopenia. In humans the disease is characterized by fever, headache, mylagia, and leukopenia. Early detection and treatment are important for treating both canine and human ***ehrlichiosis*** .

SUMM . . . are frequently used as aids in the diagnosis of these diseases. These assays measure or otherwise detect the binding of anti- ***Ehrlichia*** antibodies from a patient's blood, plasma, or serum to infected cells, cell lysates, or purified ***Ehrlichia*** proteins. However, currently known assays for detecting anti- ***Ehrlichia*** antibodies or fragments thereof are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the ***Ehrlichia*** antigen used in these tests. Highly purified reagents are needed to construct more accurate assays.

SUMM [0004] It is an object of the invention to provide reagents and methods for detecting anti- ***Ehrlichia*** ***canis*** antibodies and anti- ***Ehrlichia*** ***chaffeensis*** antibodies. This and other objects of the invention are provided by one or more of the embodiments described below.

SUMM [0007] Still another embodiment of the invention provides a method of detecting the presence of antibodies to ***Ehrlichia*** . The method comprises contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID. . . ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies to ***Ehrlichia*** , under conditions that allow polypeptide/antibody complexes to form. The polypeptide/antibody complexes are detected. The detection of polypeptide/antibody complexes is an indication that antibodies to ***Ehrlichia*** are present in the test sample.

SUMM . . . ID NO:7, and variants thereof, and instructions for use of the one or more polypeptides for the identification of an ***Ehrlichia*** infection in a mammal.

SUMM . . . packaging material comprises a label that indicates that the one or more polypeptides can be used for the identification of ***Ehrlichia*** infection in a mammal.

SUMM [0010] Even another embodiment of the invention provides a method of diagnosing an ***Ehrlichia*** infection in a mammal. The method comprises obtaining a biological sample from a mammal suspected of having an ***Ehrlichia*** infection, and contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1,. . . to form. Polypeptide/antibody complexes are detected, wherein the detection of polypeptide/antibody complexes is an indication that the mammal has an ***Ehrlichia*** infection.

SUMM [0011] Another embodiment of the invention provides a monoclonal antibody that specifically binds to at least one epitope of an ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ. . .

SUMM [0012] The invention therefore provides highly purified polypeptides and antibodies for use in accurate assays for the detection of ***Ehrlichia*** antibodies and antibody fragments.

SUMM [0014] The invention provides highly purified reagents for the detection of E. ***canis*** and E. ***chaffeensis*** antibodies and antibody fragments. In particular, the invention provides polypeptides having at least 85% identity, more preferably at least 90%. . .

SUMM [0015] The E. ***canis*** peptides were identified using phage display technology by determining the amino acid sequence bound by a mouse monoclonal antibody (IIIH7) raised against native E. ***canis*** antigen. The IIIH7 monoclonal antibody was used to affinity purify virus-expressing peptides in a PDH 10 phage display library. The sequences or mimetopes bound by IIIH7 demonstrated strong sequence homology to outer membrane proteins of E. ***canis*** and E. ***chaffeensis*** . The outer membrane proteins of both species are encoded by a polymorphic gene family, which results in multiple reproductions of the proteins.

TABLE 1

SEQ ID NO	Sequence of Peptide	Peptide Derived From
SEQ ID NO: 1	KSTVGVFGLKHDWDGSPILK	E. ***canis*** ***P30*** -1
SEQ ID NO: 2	NTTTGVFGLKQDWDGATIKD	E. ***canis***

P30

SEQ ID NO: 3 NTTVGVFGLKQNWDGSAISN E. ***chaffeensis*** P28

SEQ ID NO: 4 NPTVALYGLKQDWNGVSA E. ***chaffeensis*** OMP-1C

SEQ ID NO: 5 NTTVGVFGIEQDWDRCVIS E. ***chaffeensis*** OMP-1D

SEQ ID NO: 6 NPTVALYGLKQDWEGISS E. ***chaffeensis*** OMP-1E

SEQ ID NO: 7 NTTTGVFGLKQDWDGSTIS E. ***chaffeensis*** OMP-1F

SUMM . . . function. Thus, positions tolerating amino acid substitution may be modified while still maintaining specific binding activity of the polypeptide to anti- ***Ehrlichia*** antibodies or antibody fragments.

SUMM . . . be used (Cunningham et al., Science, 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for specific binding to anti- ***Ehrlichia*** antibodies or antibody fragments.

SUMM [0024] Polypeptides of the invention specifically bind to an anti- ***Ehrlichia*** antibody. In this context "specifically binds" means that the polypeptide recognizes and binds to an anti- ***Ehrlichia*** antibody, but does not substantially recognize and bind other molecules in a test sample.

SUMM [0025] Polypeptides of the invention comprise at least one epitope that is recognized by an anti- ***Ehrlichia*** antibody. An epitope is an antigenic determinant of a polypeptide. An epitope can be a linear, sequential epitope or a. . .

SUMM . . . are well known in the art. A polypeptide of the invention can also be produced recombinantly. A polynucleotide encoding an ***Ehrlichia*** polypeptide can be introduced into an expression vector that can be expressed in a suitable expression system using techniques well. . . expression systems are available in the art and any such expression system can be used. Optionally, a polynucleotide encoding an ***Ehrlichia*** polypeptide can be translated in a cell-free translation system.

SUMM [0027] If desired, an ***Ehrlichia*** polypeptide can be produced as a fusion protein, which can also contain other amino acid sequences, such as amino acid. . . as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. More than one ***Ehrlichia*** polypeptide can be present in a fusion protein. If desired, various combinations of ***Ehrlichia*** polypeptides from different ehrilichia strains or isolates can be included in a fusion protein.

SUMM [0028] A polypeptide of the invention can be synthesized such that it comprises several repeated ***Ehrlichia*** polypeptides. This is a multimeric polypeptide. These repeated polypeptides can comprise one

specific polypeptide, e.g. the polypeptide shown in SEQ. . . ID NO:1, repeated 2 or more times. Alternatively, the repeated polypeptides can comprise one or more copies of a specific ***Ehrlichia*** polypeptide along with one or more copies of another different ***Ehrlichia*** polypeptide. A polypeptide of the invention can be combined or synthesized with one or more polypeptides, fragments of polypeptides, or full-length polypeptides. Preferably the one or more polypeptides are other polypeptides of the invention or other ***Ehrlichia*** proteins.

SUMM [0031] Various strains and isolates of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** occur, and polypeptides of any of these strains and isolates can be used in the present invention. Nucleic acid and amino acid sequences of ***Ehrlichia*** genes and polypeptides are known in the art. For example, several sequences of the E. ***chaffeensis*** OMP gene family and several sequences of the E. ***canis*** ***P30*** gene family are disclosed in WO 99/13720.

SUMM [0033] The methods of the invention detect ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** antibodies or antibody fragments in a test sample, such as a biological sample, an environmental sample, or a laboratory sample.. . .

SUMM . . . test sample under conditions that allow a polypeptide/antibody complex to form. The formation of a complex between the polypeptide and anti- ***Ehrlichia*** antibodies in the sample is detected. In one embodiment of the invention, the polypeptide/antibody complex is detected when an indicator. . .

SUMM [0036] Polypeptides of the invention can be used to detect anti- ***Ehrlichia*** antibodies or antibody fragments in assays including, but not limited to enzyme linked immunosorbent assay (ELISA), western blot, IFA, radioimmunoassay. . .

SUMM . . . format, one or more polypeptides can be coated on a solid phase or substrate. A test sample suspected of containing anti- ***Ehrlichia*** antibodies is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody specific for ***Ehrlichia*** for a time and under conditions sufficient to form antigen/antibody complexes of either antibodies of the test sample to the polypeptides of the solid phase or the indicator reagent compound conjugated to an antibody specific for ***Ehrlichia*** to the polypeptides of the solid phase. The reduction in binding of the indicator reagent conjugated to an anti- ***Ehrlichia*** antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative ***Ehrlichia*** test sample indicates the presence of anti- ***Ehrlichia*** antibody in the test sample. This type of assay can quantitate the amount of anti- ** Ehrlichia*** antibodies in a test sample.

SUMM . . . to an indicator reagent and added to a test sample. This mixture is applied to the support or substrate. If ***Ehrlichia*** antibodies are present in the test sample they will bind the polypeptide conjugated to an indicator reagent and to the. . . immobilized on the support. The polypeptide/antibody/indicator complex can then be detected. This type of assay can quantitate the amount of anti-***Ehrlichia*** antibodies in a test sample.

SUMM [0040] Formation of the complex is indicative of the presence of anti-E. ***canis*** or anti-E. ***chaffeensis*** antibodies in a test sample. Therefore, the methods of the invention can be used to diagnose E. ***canis*** or E. ***chaffeensis*** infection in a patient. Each polypeptide of the invention can detect E. ***canis*** or E. ***chaffeensis*** or both due to cross-reactivity of the polypeptides and antibodies.

SUMM [0041] The methods of the invention can also indicate the amount or quantity of anti- ***Ehrlichia*** antibodies in a test sample. With many indicator reagents, such as enzymes, the amount of antibody present is proportional to. . .

SUMM [0042] The invention further comprises assay kits for detecting anti-***Ehrlichia*** antibodies in a sample. A kit comprises one or more polypeptides of the invention and means for determining binding of the polypeptide to ***Ehrlichia*** antibodies in the sample. A kit can comprise a device containing one or more polypeptides of the invention and instructions for use of the one or more polypeptides for the identification of an ***Ehrlichia*** infection in a mammal. The kit can also comprise packaging material comprising a label that indicates that the one or more polypeptides of the kit can be used for the identification of ***Ehrlichia*** infection. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, may be. . . kits. The polypeptides, assays, and kits of the invention are useful, for example, in the diagnosis of individual cases of ***Ehrlichia*** infection in a patient, as well as epidemiological studies of ***Ehrlichia*** outbreaks.

SUMM [0043] Polypeptides and assays of the invention can be combined with other polypeptides or assays to detect the presence of ***Ehrlichia*** along with other organisms. For example, polypeptides and assays of the invention can be combined with reagents that detect heartworm. . .

SUMM . . . invention can also be used to develop monoclonal and/or polyclonal antibodies that specifically bind to an immunological epitope of E. ***canis*** or E. ***chaffeensis*** present in the polypeptides of the invention.

SUMM . . . binding assay, enzyme linked immunosorbent assay, western blot assay, or indirect immunofluorescense assay, to determine the presence, if any, of ***Ehrlichia*** polypeptides in a test sample. In addition, these antibodies, in particular monoclonal antibodies, can be

bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific ***Ehrlichia*** proteins from, for example, cell cultures or blood serum, such as to purify recombinant and native ***Ehrlichia*** antigens and proteins. The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for. . .

SUMM [0047] Monoclonal antibodies directed against ***Ehrlichia*** epitopes can be produced by one skilled in the art. The general methodology for producing such antibodies is well-known and. . .

DETD [0049] Detection of E. ***canis*** Antibodies in Canine Serum

DETD [0050] The performance of a synthetic peptide SNAP.RTM. assay was compared to the performance of a commercially available E. ***canis*** SNAP.RTM. assay that uses partially purified E. ***canis*** antigens. The partially purified native antigens were obtained from E. ***canis*** organisms grown in tissue culture and partially purified by differential centrifugation and column chromatography. The synthetic peptides used in the synthetic peptide SNAP.RTM. assay were monomeric forms of the E. ***canis*** ***P30*** -1 or the E. ***canis*** P-30 peptide, SEQ ID NO:1 and SEQ ID NO:2, respectively.

DETD [0051] A population of 70 suspected E. ***canis*** positive canine samples was obtained from Arizona, Texas, and Arkansas and tested using the synthetic peptide SNAP.RTM. assay and the. . . antigen SNAP.RTM. assay. The samples were also tested using an indirect IFA. Briefly, the IFA assay was performed using E. ***canis*** infected cells coated onto IFA slides and fluorescein isothiocyanate (FITC)-labeled rabbit anti-canine IgG. E. ***canis*** was harvested from cell cultures, diluted in buffer and coated onto IFA slides. Dilutions of test samples were made in. . .

DETD [0052] In the case of discrepant results, an E. ***canis*** western blot was used as the confirmatory assay. Briefly, E. ***canis*** antigen was harvested from tissue culture, resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose membrane. After transfer, the membrane was blocked with heterologous protein overnight at 4 degrees C. Diluted test samples of canine E. ***canis*** Ab-positive and negative serum samples were incubated with blots for 2 hours at room temperature. Blots were then washed, incubated. . .

DETD . . . assay device and allowed to flow along and saturate a flow matrix. This facilitates sequential complex formation. That is, an ***Ehrlichia*** antibody in the test sample binds first to an non-immobilized labeled specific binding reagent. In the case of the synthetic. . .

DETD . . . (3/5). Therefore, the synthetic peptide SNAP.RTM. assay is more sensitive and specific than the native antigen SNAP.RTM. assay.

TABLE 2

E. ***canis*** Ab Positive Canine Population Comparison of Native Antigen SNAP Assay with Synthetic Peptide SNAP Assay

	Native Ag Assay	Synthetic Peptide Assay	E. ***canis*** IFA Western	
No.	Sample I.D.	E. ***canis*** H. Worm	E. ***canis*** H. Worm	Titer .gtoreq. Blot
1	F119894-6	- -	- -	1:100 -
2	F103638-5	- -	- -	1:100 -
3	2815:89E. . .			

CLM What is claimed is:

3. A method of detecting presence of antibodies to ***Ehrlichia*** comprising: (a) contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1,. . . ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies to ***Ehrlichia*** , under conditions that allow polypeptide/antibody complexes to form; (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that antibodies to ***Ehrlichia*** are present in the test sample.

5. The method of claim 3, wherein the presence of antibodies to ***Ehrlichia*** ***canis*** are detected.

6. The method of claim 3, wherein the presence of antibodies to ***Ehrlichia*** ***chaffeensis*** are detected.

. . . device of claim 21, further comprising instructions for use of the one or more polypeptides for the identification of an ***Ehrlichia*** infection in a mammal.

23. The device of claim 22, wherein the identification of an ***Ehrlichia*** infection is done using a method of detecting presence of antibodies to ***Ehrlichia*** comprising: (a) contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1,. . . ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies

to ***Ehrlichia*** , under conditions that allow polypeptide/antibody complexes to form; (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that an ***Ehrlichia*** infection is present.

24. The device of claim 22, wherein the ***Ehrlichia*** infection is caused by ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** .

26. The article of manufacture of claim 25 wherein the packaging material comprises a label that indicates that the one or more polypeptides can be used for the identification of ***Ehrlichia*** infection in a mammal.

27. The article of manufacture of claim 26, wherein the identification of an ***Ehrlichia*** infection is done using a method of detecting presence of antibodies to ***Ehrlichia*** comprising: (a) contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1,. . . ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies to ***Ehrlichia*** , under conditions that allow polypeptide/antibody complexes to form; (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that an ***Ehrlichia*** infection is present.

28. The article of manufacture of claim 26, wherein the ***Ehrlichia*** infection is caused by ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** .

29. A method of diagnosing an ***Ehrlichia*** infection in a mammal comprising: (a) obtaining a biological sample from a mammal suspected of having an ***Ehrlichia*** infection; (b) contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1,. . . to form; (c) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the mammal has an ***Ehrlichia*** infection.

31. The method of claim 29, wherein the ***Ehrlichia*** infection is caused by ***Ehrlichia*** ***canis*** .

32. The method of claim 29, wherein the ***Ehrlichia*** infection is caused by ***Ehrlichia*** ***chaffeensis*** .

34. A monoclonal antibody that specifically binds to at least one epitope of an ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** polypeptide, said polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID. . .

L7 ANSWER 13 OF 30 USPATFULL
AN 2002:287569 USPATFULL
TI Compositions and methods for detection of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies
IN Lawton, Robert, Gorham, ME, UNITED STATES
O'Connor, Thomas Patrick, JR., Westbrook, ME, UNITED STATES
Bartol, Barbara Ann, Gorham, ME, UNITED STATES
MacHenry, Paul Scott, Portland, ME, UNITED STATES
PA IDEXX Laboratories. (U.S. corporation)
PI US 2002160432 A1 20021031
AI US 2002-54647 A1 20020122 (10)
RLI Division of Ser. No. US 2001-765739, filed on 18 Jan 2001, PENDING
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SUMM [0001] The invention provides compositions and methods for the detection and quantification of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** antibodies and antibody fragments.
SUMM [0002] The ***Ehrlichia*** are obligate intracellular pathogens that infect circulating lymphocytes in mammalian hosts. ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** are members of the same sub-genus group that infect canines and humans and cause canine monocytic ***ehrlichiosis*** (CME) and human monocytic ***ehrlichiosis*** (HME), respectively. The canine disease is

characterized by fever, lymphadenopathy, weight loss, and pancytopenia. In humans the disease is characterized by fever, headache, mylagia, and leukopenia. Early detection and treatment are important for treating both canine and human ***ehrlichiosis*** .

SUMM . . . are frequently used as aids in the diagnosis of these diseases. These assays measure or otherwise detect the binding of anti- ***Ehrlichia*** antibodies from a patient's blood, plasma, or serum to infected cells, cell lysates, or purified ***Ehrlichia*** proteins. However, currently known assays for detecting anti- ***Ehrlichia*** antibodies or fragments thereof are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the ***Ehrlichia*** antigen used in these tests. Highly purified reagents are needed to construct more accurate assays.

SUMM [0004] It is an object of the invention to provide reagents and methods for detecting anti- ***Ehrlichia*** ***canis*** antibodies and anti- ***Ehrlichia*** ***chaffeensis*** antibodies. This and other objects of the invention are provided by one or more of the embodiments described below.

SUMM [0007] Still another embodiment of the invention provides a method of detecting the presence of antibodies to ***Ehrlichia*** . The method comprises contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID. . . ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies to ***Ehrlichia*** , under conditions that allow polypeptide/antibody complexes to form. The polypeptide/antibody complexes are detected. The detection of polypeptide/antibody complexes is an indication that antibodies to ***Ehrlichia*** are present in the test sample.

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SUMM [0011] Another embodiment of the invention provides a monoclonal antibody that specifically binds to at least one epitope of an ***Ehrlichia*** ***canis*** or ***Ehrlichia***

chaffeensis polypeptide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4,. . .

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SUMM [0015] The E. ***canis*** peptides were identified using phage display technology by determining the amino acid sequence bound by a mouse monoclonal antibody (IIIH7) raised against native E. ***canis*** antigen. The IIIH7 monoclonal antibody was used to affinity purify virus-expressing peptides in a PDH 10 phage display library. The sequences or mimetopes bound by IIIH7 demonstrated strong sequence homology to outer membrane proteins of E. ***canis*** and E. ***chaffeensis*** . The outer membrane proteins of both species are encoded by a polymorphic gene family, which results in multiple reproductions of the proteins.

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SEQ ID NO:2	NTTTGVFGLKQDWDGATIKD	E. ***canis*** ***P30***
SEQ ID NO:3	NTTVGVFGLKQNWDGSAISN	E. ***chaffeensis*** P28
SEQ ID NO:4	NPTVALYGLKQDWNGVSA	E. ***chaffeensis*** OMP-1C
SEQ ID NO:5	NTTVGVFGIEQDWDRCVIS	E. ***chaffeensis*** OMP-1D
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SUMM [0027] If desired, an ***Ehrlichia*** polypeptide can be produced as a fusion protein, which can also contain other amino acid sequences, such as amino acid. . . as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. More than one ***Ehrlichia*** polypeptide can be present in a fusion protein. If desired, various combinations of ***Ehrlichia*** polypeptides from different ehrilichia strains or isolates can be included in a fusion protein.

SUMM [0028] A polypeptide of the invention can be synthesized such that it comprises several repeated ***Ehrlichia*** polypeptides. This is a multimeric polypeptide. These repeated polypeptides can comprise one specific polypeptide, e.g. the polypeptide shown in SEQ. . . ID NO: 1, repeated 2or more times. Alternatively, the repeated polypeptides can comprise one or more copies of a specific ***Ehrlichia*** polypeptide along with one or more copies of another different ***Ehrlichia*** polypeptide. A polypeptide of the invention can be combined or synthesized with one or more polypeptides, fragments of polypeptides, or full-length polypeptides. Preferably the one or more polypeptides are other polypeptides of the invention or other ***Ehrlichia*** proteins.

SUMM [0031] Various strains and isolates of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** occur, and polypeptides of

any of these strains and isolates can be used in the present invention. Nucleic acid and amino acid sequences of ***Ehrlichia*** genes and polypeptides are known in the art. For example, several sequences of the E. ***chaffeensis*** OMP gene family and several sequences of the E. ***canis*** ***P30*** gene family are disclosed in WO 99/13720.

SUMM [0033] The methods of the invention detect ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** antibodies or antibody fragments in a test sample, such as a biological sample, an environmental sample, or a laboratory sample.. . .

SUMM . . . test sample under conditions that allow a polypeptide/antibody complex to form. The formation of a complex between the polypeptide and anti- ***Ehrlichia*** antibodies in the sample is detected. In one embodiment of the invention, the polypeptide/antibody complex is detected when an indicator. . .

SUMM [0036] Polypeptides of the invention can be used to detect anti- ***Ehrlichia*** antibodies or antibody fragments in assays including, but not limited to enzyme linked immunosorbent assay (ELISA), western blot, IFA, radioimmunoassay. . .

SUMM . . . format, one or more polypeptides can be coated on a solid phase or substrate. A test sample suspected of containing anti- ***Ehrlichia*** antibodies is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody specific for ***Ehrlichia*** for a time and under conditions sufficient to form antigen/antibody complexes of either antibodies of the test sample to the polypeptides of the solid phase or the indicator reagent compound conjugated to an antibody specific for ***Ehrlichia*** to the polypeptides of the solid phase. The reduction in binding of the indicator reagent conjugated to an anti- ***Ehrlichia*** antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative ***Ehrlichia*** test sample indicates the presence of anti- ***Ehrlichia*** antibody in the test sample. This type of assay can quantitate the amount of anti- ***Ehrlichia*** antibodies in a test sample.

SUMM . . . to an indicator reagent and added to a test sample. This mixture is applied to the support or substrate. If ***Ehrlichia*** antibodies are present in the test sample they will bind the polypeptide conjugated to an indicator reagent and to the. . . immobilized on the support. The polypeptide/antibody/indicator complex can then be detected. This type of assay can quantitate the amount of anti- ***Ehrlichia*** antibodies in a test sample.

SUMM [0040] Formation of the complex is indicative of the presence of anti-E. ***canis*** or anti-E. ***chaffeensis*** antibodies in a test sample. Therefore, the methods of the invention can be used to diagnose E. ***canis*** or E. * *chaffeensis*** infection in a patient.

Each polypeptide of the invention can detect E. ***canis*** or E. ***chaffeensis*** or both due to cross-reactivity of the polypeptides and antibodies.

SUMM [0041] The methods of the invention can also indicate the amount or quantity of anti- ***Ehrlichia*** antibodies in a test sample. With many indicator reagents, such as enzymes, the amount of antibody present is proportional to. . .

SUMM [0042] The invention further comprises assay kits for detecting anti- ***Ehrlichia*** antibodies in a sample. A kit comprises one or more polypeptides of the invention and means for determining binding of the polypeptide to ***Ehrlichia*** antibodies in the sample. A kit can comprise a device containing one or more polypeptides of the invention and instructions for use of the one or more polypeptides for the identification of an ***Ehrlichia*** infection in a mammal. The kit can also comprise packaging material comprising a label that indicates that the one or more polypeptides of the kit can be used for the identification of ***Ehrlichia*** infection. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, may be. . . kits. The polypeptides, assays, and kits of the invention are useful, for example, in the diagnosis of individual cases of ***Ehrlichia*** infection in a patient, as well as epidemiological studies of ***Ehrlichia*** outbreaks.

SUMM [0043] Polypeptides and assays of the invention can be combined with other polypeptides or assays to detect the presence of ***Ehrlichia*** along with other organisms. For example, polypeptides and assays of the invention can be combined with reagents that detect heartworm. . .

SUMM . . . invention can also be used to develop monoclonal and/or polyclonal antibodies that specifically bind to an immunological epitope of E. ***canis*** or E. ***chaffeensis*** present in the polypeptides of the invention.

SUMM . . . binding assay, enzyme linked immunosorbent assay, western blot assay, or indirect immunofluorescense assay, to determine the presence, if any, of ***Ehrlichia*** polypeptides in a test sample. In addition, these antibodies, in particular monoclonal antibodies, can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific ***Ehrlichia*** proteins from, for example, cell cultures or blood serum, such as to purify recombinant and native ***Ehrlichia*** antigens and proteins. The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for. . .

SUMM [0047] Monoclonal antibodies directed against ***Ehrlichia*** epitopes can be produced by one skilled in the art. The general methodology for producing such antibodies is well-known and. . .

DETD [0049] Detection of E. ***canis*** Antibodies in Canine Serum

DETD [0050] The performance of a synthetic peptide SNAP.RTM. assay was

compared to the performance of a commercially available E. ***canis*** SNAP.RTM. assay that uses partially purified E. ***canis*** antigens. The partially purified native antigens were obtained from E. ***canis*** organisms grown in tissue culture and partially purified by differential centrifugation and column chromatography. The synthetic peptides used in the synthetic peptide SNAP.RTM. assay were monomeric forms of the E. ***canis*** ***P30*** -1 or the E. ***canis*** P-30 peptide, SEQ ID NO:1 and SEQ ID NO:2, respectively.

DETD [0051] A population of 70 suspected E. ***canis*** positive canine samples was obtained from Arizona, Texas, and Arkansas and tested using the synthetic peptide SNAP.RTM. assay and the. . . . antigen SNAP.RTM. assay. The samples were also tested using an indirect IFA. Briefly, the IFA assay was performed using E. ***canis*** infected cells coated onto IFA slides and fluorescein isothiocyanate (FITC)-labeled rabbit anti-canine IgG. E. ***canis*** was harvested from cell cultures, diluted in buffer and coated onto IFA slides. Dilutions of test samples were made in. . .

DETD [0052] In the case of discrepant results, an E. ***canis*** western blot was used as the confirmatory assay. Briefly, E. ***canis*** antigen was harvested from tissue culture, resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose membrane. After transfer, the membrane was blocked with heterologous protein overnight at 4 degrees C. Diluted test samples of canine E. ***canis*** Ab-positive and negative serum samples were incubated with blots for 2 hours at room temperature. Blots were then washed, incubated. . .

DETD . . . assay device and allowed to flow along and saturate a flow matrix. This facilitates sequential complex formation. That is, an ***Ehrlichia*** antibody in the test sample binds first to an non-immobilized labeled specific binding reagent. In the case of the synthetic. . .

DETD . . . (3/5). Therefore, the synthetic peptide SNAP.RTM. assay is more sensitive and specific than the native antigen SNAP.RTM. assay.

TABLE 2

E. ***canis*** Ab Positive Canine Population Comparison of Native Antigen SNAP Assay with Synthetic Peptide SNAP Assay

		Native Ag Assay	Synthetic Peptide Assay	E. ***Canis***	Western
No.	Sample I.D.	E. ***Canis***	H. Worm	E. ***Canis **	H.
		291JS	358HT & 359HT	IFA	

Worm Titer .gtoreq. Blot

1 F119894-6 - - - - 1:100
-
2 F103638-5 - - - - 1:100
-
3 2815:89E. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 1
LENGTH: 20
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 1
Lys Ser Thr Val Gly Val Phe Gly Leu Lys His Asp Trp Asp Gly Ser
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 2
LENGTH: 20
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***canis***
SEQUENCE: 2
Asn Thr Thr Thr Gly Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ala
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 20
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 3
Asn Thr Thr Val Gly Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 18
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 4
Asn Pro Thr Val Ala Leu Tyr Gly Leu Lys Gln Asp Trp Asn Gly Val
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 19
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 5

Asn Thr Thr Val Gly Val Phe Gly Ile Glu Gln Asp Trp Asp Arg Cys
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 18
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 6
Asn Pro Thr Val Ala Leu Tyr Gly Leu Lys Gln Asp Trp Glu Gly Ile
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7
LENGTH: 19
TYPE: PRT
ORGANISM: ***Ehrlichia*** ***chaffeensis***
SEQUENCE: 7
Asn Thr Thr Thr Gly Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ser
1 5 10. . .
CLM What is claimed is:
. . . 3 wherein the packaging material comprises a label that indicates that the polypeptide can be used for the identification of ***Ehrlichia*** infection in a mammal.

5. The article of manufacture of claim 4, wherein the identification of an ***Ehrlichia*** infection is done using a method of detecting presence of antibodies to ***Ehrlichia*** comprising: (a) contacting a polypeptide shown in SEQ ID NO:2, or variants thereof, with a test sample suspected of comprising antibodies to ***Ehrlichia*** , under conditions that allow polypeptide/antibody complexes to form; (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that an ***Ehrlichia*** infection is present.

6. The article of manufacture of claim 4, wherein the ***Ehrlichia*** infection is caused by ***Ehrlichia*** ***canis*** or ***Ehrlichia*** ***chaffeensis*** .

L7 ANSWER 14 OF 30 USPATFULL
AN 2002:214448 USPATFULL
TI Homologous 28-kilodaltion immunodominant protein genes of ***Ehrlichia*** ***canis*** and uses thereof
IN Walker, David H., Galveston, TX, UNITED STATES
Yu, Xue-Jie, Houston, TX, UNITED STATES
McBride, Jere W., Galveston, TX, UNITED STATES

PI US 2002115840 A1 20020822
AI US 2002-62624 A1 20020131 (10)
RLI Division of Ser. No. US 2000-660279, filed on 12 Sep 2000, PENDING Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999, GRANTED, Pat. No. US 6403780 Continuation-in-part of Ser. No. US 1998-201458, filed on 30 Nov 1998, ABANDONED
DT Utility
FS APPLICATION
LREP Benjamin Aaron Adler, Ph.D., J.D.; Adler & Associates, 8011 Candle Lane, Houston, TX, 77071
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 20 Drawing Page(s)
LN.CNT 2267
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.
TI Homologous 28-kilodaltion immunodominant protein genes of ***Ehrlichia*** ***canis*** and uses thereof
AB . . . of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and. . .
SUMM . . . of molecular biology. More specifically, the present invention relates to molecular cloning and characterization of homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , a multigene locus encoding the 28-kDa homologous proteins of ***Ehrlichia*** ***canis*** and uses thereof.
SUMM [0005] Canine ***ehrlichiosis*** , also known as canine tropical pancytopenia, is a tick-borne rickettsial disease of dogs first described in Africa in 1935 and. . . outbreak occurred in United States military dogs during the Vietnam War (Walker et al., 1970) The

etiologic agent of canine ***ehrlichiosis*** is ***Ehrlichia*** ***canis*** , a small, gram-negative, obligate intracellular bacterium which exhibits tropism for mononuclear phagocytes (Nyindo et al., 1971) and is transmitted by the brown dog tick, Rhipicephalus sanguineus (Groves et al., 1975). The progression of canine ***ehrlichiosis*** occurs in three phases, acute, subclinical and chronic. The acute phase is characterized by fever, anorexia, depression, lymphadenopathy and mild. . .

SUMM . . . persistent infections in the host. Although disease pathogenesis is poorly understood, multigene families described in members of the related genera ***Ehrlichia*** , Anaplasma, and Cowdria may be involved in variation of major surface antigen expression thereby evading immune surveillance. Anaplasma marginale, an organism closely related to E ***canis*** , exhibits variation of major surface protein 3 (msp-3) genes resulting in antigenic polymorphism among strains (Alleman et al., 1997).

SUMM [0007] Molecular taxonomic analysis based on the 16S rRNA gene has determined that E. ***canis*** and E. ***chaffeensis*** , the etiologic agent of human monocytic ***ehrlichiosis*** (HME), are closely related (Anderson et al., 1991; Anderson et al., 1992; Dawson et al., 1991; Chen et al., 1994). Considerable cross reactivity of the 64, 47, 40, 30, 29 and 23-kDa antigens between E. ***canis*** and E. ***chaffeensis*** has been reported (Chen et al., 1994; Chen et al., 1997; Rikihisa et al., 1994; Rikihisa et al., 1992). Analysis. . . with human and canine convalescent phase sera by immunoblot has resulted in the identification of numerous immunodominant proteins of E. ***canis*** , including a 30-kDa protein (Chen et al., 1997). In addition, a 30-kDa protein of E. ***canis*** has been described as a major immunodominant antigen recognized early in the immune response that is antigenically distinct from the 30-kDa protein of E. ***chaffeensis*** (Rikihisa et al., 1992; Rikihisa et al., 1994). Other immunodominant proteins of E. ***canis*** with molecular masses ranging from 20 to 30-kDa have also been identified (Brouqui et al., 1992; Nyindo et al., 1991;. . .

SUMM [0008] Homologous 28-32kDa immunodominant proteins encoded by multigene families have been reported in related organisms including, E. ***chaffeensis*** and Cowdria ruminantium (Sulsona et al., 1999; Ohashi et al., 1998a; Reddy et al., 1998). Recently, characterization of a 21 member multigene family encoding proteins of 23 to 28-kDa has been described in E. ***chaffeensis*** (Yu et al., 2000). The E. ***chaffeensis*** 28-kDa outer membrane proteins are surface exposed, and contain three major hypervariable regions (Ohashi et al., 1998a). The recombinant E. ***chaffeensis*** P28 appeared to provide protection against homologous challenge infection in mice, and antisera produced against the recombinant protein cross reacted with a 30-kDa

protein of E. ***canis*** (Ohashi et al., 1998a). Diversity in the p28 gene among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a), and studies using monoclonal antibodies have further demonstrated diversity in the expressed P28 proteins (Yu et al., 1993). Conversely, complete conservation of a p28 genes in geographically different isolates of E. ***canis*** has been reported and suggests that E. ***canis*** may be conserved in North America (McBride et al., 1999, 2000).

SUMM . . . prior art is deficient in the lack of cloning an d characterization of new homologous 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** and a single multigene locus containing the homologous 28-kDa protein genes. Further, The prior art is deficient in the lack of recombinant proteins of such immunoreactive genes of ***Ehrlichia*** ***canis*** . The present invention fulfills this long-standing need and desire in the art.

SUMM . . . of the present invention describe the molecular cloning, sequencing, characterization, and expression of homologous mature 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** (designated p28-1; -2, -3, -5, -6, -7, -9), and the identification of a single locus (10,677-bp) containing nine 28-kDa protein genes of ***Ehrlichia*** ***canis*** (p28-1 to p28-9). Eight of the p28 genes were located on one DNA strand, and one p28 gene was found. . .

SUMM [0011] In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

SUMM . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

SUMM [0015] The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through . any means such as, e.g., the stimulation of the subject's humoral or cellular. . .

DRWD . . . arrow) and 16-kDa thioredoxin control (Lane 2, arrow), and corresponding immunoblot of recombinant p28-7-thioredoxin fusion protein recognized by covalescent-phase E. ***canis*** canine antiserum

(Lane 3). Thiroredoxin control was not detected by E. ***canis*** antiserum (not shown).

DRWD . . . ID NO. 2), p28-5 protein (ECa28SA2, partial sequence, SEQ ID NO. 7), p28-4 protein (ECa28SA1, SEQ ID NO. 8), E. ***chaffeensis*** P28 (SEQ ID NO. 9), E. ***chaffeensis*** OMP-1 family (SEQ ID NOs: 10-14) and C. ruminantium MAP-1 protein (SEQ ID NO. 15). The p28-7 amino acid sequence. . .

DRWD [0021] FIG. 4 shows phylogenetic relatedness of E. ***canis*** p28-7 (ECa28-1), p28-5 (ECa28SA2, partial sequence), p28-4 (ECa28SA1), members of the E. ***chaffeensis*** omp-1 multiple gene family, and C. rumanintium map-1 protein from deduced amino acid sequences utilizing unbalanced tree construction. The length. . .

DRWD [0022] FIG. 5 shows Southern blot analysis of E. ***canis*** genomic DNA completely digested with six individual restriction enzymes and hybridized with a p28-7 DIG-labeled probe (Lanes 2-7); DIG-labeled molecular. . .

DRWD [0023] FIG. 6 shows comparison of predicted protein characteristics of E. ***canis*** p28-7 (ECa28-1, Jake strain) and E. ***chaffeensis*** P28 (Arkansas strain). Surface probability predicts the surface residues by using a window of hexapeptide. A surface residue is any. . .

DRWD [0024] FIG. 7 shows nucleic acid sequences and deduced amino acid sequences of the E. ***canis*** 28-kDa protein genes p28-5 (nucleotide 1-849: SEQ ID No. 3; amino acid sequence: SEQ ID No. 4) and p28-6 (nucleotide. . .

DRWD [0025] FIG. 8 shows schematic of the E. ***canis*** 28-kDa protein gene locus (5.592-Kb, containing five genes) indicating genomic orientation and intergenic noncoding regions (28NC1-4). The 28-kDa protein genes. . .

DRWD [0026] FIG. 9 shows phylogenetic relatedness of the E. ***canis*** 28-kDa protein gene p28-4 (ECa28SA1), p.sup.28-5 (ECa28SA2), p28-6 (ECa28SA3), p28-7 (ECa28-1-) and p28-8 (ECa28-2) based on amino acid sequences utilizing. . .

DRWD [0027] FIG. 10 shows alignment of E. ***canis*** 28-kDa protein gene intergenic noncoding nucleic acid sequences (SEQ ID Nos. 30-33). Nucleic acids not shown, denoted with a dot. . .

DRWD [0028] FIG. 11 shows schematic representation of the nine gene E. ***canis*** p28 locus (10,677-bp) indicating genomic orientation and intergenic noncoding regions. The p28 genes (p28-1, 2, 3, 9) (unshaded) were identified in Example 8. Shaded p28 genes have been identified previously and designated as follows: p28-4, ***p30a*** (Ohashi et al., 1998b) and ORF1 (Reddy et al., 1998); p28-5 and p28-6, (McBride, et. al., 2000); p28-7, p2.sup.8 (McBride et al., 1999) and ***p30*** (Ohashi et al., 1998b); and p28-8, ***p30*** -1 (Ohashi et al., 1998b).

DRWD [0029] FIG. 12 shows phylogenetic relationships of E. ***canis*** P28-1 to P28-9 based on the amino acid sequences. The length of each pair of branches represents the distance between. . .

DRWD . . . 13 shows nucleic acid sequence (SEQ ID No. 39) and deduced amino acid sequence (SEQ ID No. 40) of E. ***canis*** p28-1 gene.

DRWD . . . 14 shows nucleic acid sequence (SEQ ID No. 41) and deduced amino acid sequence (SEQ ID No. 42) of E. ***canis*** p28-2 gene.

DRWD . . . 15 shows nucleic acid sequence (SEQ ID No. 43) and deduced amino acid sequence (SEQ ID No. 44) of E. ***canis*** p28-3 gene.

DRWD . . . 16 shows nucleic acid sequence (SEQ ID No. 45) and deduced amino acid sequence (SEQ ID No. 46) of E. ***canis*** p28-9 gene.

DETD [0034] The present invention describes cloning, sequencing and expression of homologous genes encoding a 30-kilodalton (kDa) protein of ***Ehrlichia*** ***canis*** . A comparative molecular analysis of homologous genes among seven E. ***canis*** isolates and the E. ***chaffeensis*** omp-1 multigene family was also performed. Several new 28-kDa protein genes are identified as follows:

DETD [0037] Using PCR to amplify 28-kDa protein genes of E. ***canis*** , a previously unsequenced region of p28-5 (Eca28SA2) was completed. Sequence analysis of p28-5 revealed an 849-bp open reading frame encoding. . .

DETD . . . of tandemly arranged p28 genes were sequenced, and p28-1, -2, -3, and -9 were identified. Consequently, a nine gene E. ***canis*** p28 locus spanning 10, 677 bp was identified in the present invention.

DETD [0040] The present invention is directed to, inter alia, homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , p28-1, -2, -3, -6, -7, and p28-9, and a complete sequence of previously partially sequenced p28-5. Also disclosed is a multigene locus encoding nine homologous 28-kDa outer membrane proteins of ***Ehrlichia*** ***canis*** . Eight of the p28 genes were located on one DNA strand, and one p28 gene was found on the complementary. . .

DETD [0042] The invention includes a substantially pure DNA encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably. . .

DETD . . . listed in SEQ ID No 1, 3, 5, 39, 41, 43, or 45 which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** .

DETD . . . comprises a vector comprising a DNA sequence coding for a which encodes a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and said vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication;. . .

DETD . . . or viral nucleic acid. Vectors may be used to amplify and/or express nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . An expression vector is a replicable

construct in which a nucleic acid sequence encoding a polypeptide is operably linked to. . .

DETD . . . such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention can be used to transform a host using any of the techniques commonly known to those. . . Especially preferred is the use of a vector containing coding sequences for a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention for purposes of prokaryote transformation.

DETD . . . "recombinant" cell is intended to refer to a cell into which a recombinant gene, such as a gene encoding an ***Ehrlichia*** ***canis*** antigen has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced. . .

DETD [0052] The present invention is also drawn to substantially pure 28-30 kDa immunoreactive proteins of E. ***canis*** comprise of amino acid sequences listed in, for example, SEQ ID No. 2, 4, 6, 40, 42, 44, or 46.

DETD . . . more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** ; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography such as immunoaffinity chromatography using an antibody specific for a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from. . .

DETD . . . In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (SEQ ID No. 2, 4, 6, 40, 42, 44, or 46). As used herein, "fragment," as applied to a polypeptide,. . . 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , by recombinant DNA techniques using an expression vector that encodes a defined fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of 28-kDa immunoreactive protein of * *Ehrlichia*** ***canis*** (e.g., binding to an antibody specific

for 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis***) can be assessed by methods described herein.

DETD [0055] Purified 28-kDa immunoreactive protein of ** Ehrlichia*** ***canis*** or antigenic fragments of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay). . .

DETD [0058] Included in this invention are polyclonal antisera generated by using 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** or a fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this. . . art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability t o identify recombinant ***Ehrlichia*** ***canis*** cDNA clones, and to distinguish them from known cDNA clones.

DETD [0066] In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

DETD . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

DETD [0070] The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, i.e. the stimulation of the subject's humoral or cellular immune. . .

DETD [0073] ***Ehrlichiae*** and Purification ***Ehrlichia*** ***canis*** (Florida strain and isolates Demon, DJ, Jake, and Fuzzy) were provided by Dr. Edward Breitschwerdt, (College of Veterinary Medicine, North Carolina State University, Raleigh, N.C.). E. ***canis*** (Louisiana strain) was provided by Dr. Richard E. Corstvet (School of Veterinary Medicine, Louisiana State University, Baton Rouge, La.) and E. ***canis*** (Oklahoma strain) was provided by Dr. Jacqueline Dawson (Centers for Disease Control and Prevention, Atlanta, Ga.). Propagation of chrlichiae was. . . serum and 2 mM L-glutamine

at 37.degree. C. The intracellular growth in DH82 cells was monitored by presence of E. ***canis*** morulae using general cytologic staining methods. Cells were harvested when 100% of the cells were infected with ***ehrlichiae*** and were then pelleted in a centrifuge at 17,000.times. g for 20 min. Cell pellets were disrupted with a Braun-Sonic 2000 sonicator twice at 40W for 30 sec on ice. ***Ehrlichiae*** were purified as described previously (Weiss et al., 1975). The lysate was loaded onto discontinuous gradients of 42%-36%-30% renografin, and centrifuged at 80,000.times. g for 1 hr. Heavy and light bands containing ***ehrlichiae*** were collected and washed with sucrose-phosphate-glutamate buffer (SPG, 218 mM sucrose, 3.8 mM KH.sub.2PO.sub.4, 7.2 mM K.sub.2HP0.sub.4, 4.9 mM glutamate,. . .

DETD [0075] ***Ehrlichia*** ***canis*** genomic DNA was prepared by resuspending the renografin-purified ***ehrlichiae*** in 600 .mu.l of 10 mM Tris-HCl buffer (pH 7.5) with 1% sodium dodecyl sulfate (SDS, w/v) and 100 ng/ml. . .

DETD . . . determined using a Universal GenomeWalker Kit (CLONTECH, Palo Alto, Calif.) according to the protocol supplied by the manufacturer. Genomic E. ***canis*** (Jake isolate) DNA was digested completely with five restriction enzymes (DraI, EcoRV, PvuII, ScaI, StuI) which produce blunt-ended DNA. An adapter (AP1) supplied in the kit was ligated to each end of E. ***canis*** DNA. The genomic libraries were used as templates to find the unknown DNA sequence of the p28-7 gene by PCR. . .

DETD . . . with an ABI Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The entire p28-7 genes of seven E. ***canis*** isolates (four from North Carolina, and one each from Oklahoma, Florida, and Louisiana) were amplified by PCR with primers EC28OM-F. . .

DETD [0080] PCR Amplification, Cloning, Sequencing and Expression of E. ***canis*** ECa28/1 (p28-7) Gene

DETD [0082] The entire E. ***canis*** p28-7 gene was PCR-amplified with primers-EC28OM-F and EC28OM-R and cloned into pCR2.1-TOPO TA cloning vector to obtain the desired set. . .

DETD [0084] Recombinant E. ***canis*** p28-7 fusion protein was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-15% Tris-HCl gradient gels (Bio-Rad, Hercules, Calif.) and transferred. . . N.H.) using a semi-dry transfer cell (Bio-Rad, Hercules, Calif.). The membrane was incubated with convalescent phase antisera from an E. ***canis*** -infected dog diluted 1:5000 for 1 hour, washed, and then incubated with an anti-canine IgG (H & L) alkaline phosphatase-conjugated affinity-purified. . .

DETD [0086] To determine if multiple genes homologous to the p28-7 gene were present in the E. ***canis*** genome, a genomic Southern blot analysis was performed using a standard procedure (Sambrook et al.

1989). E. ***canis*** genomic DNA digested completely with each of the restriction enzymes BanII, EcoRV, HaeII, KpnI and SpeI, which do not cut. . . digested probe (566-bp) was separated by agarose gel electrophoresis, gel-purified and then used for hybridization. The completely digested genomic E. ***canis*** DNA was electrophoresed and transferred to a nylon membrane (Boehringer Mannheim, Indianapolis, Ind.) and hybridized at 40.degree. C. for 16. . .

DETD [0088] E. ***chaffeensis*** p28 and C. ruminantium map-1 DNA sequences were obtained from the National Center of Biotechnology Information (NCBI). Nucleotide and deduced. . .

DETD [0089] Sequence analysis of p28-7 from seven different strains of E. ***canis*** was performed with primers designed to amplify the entire gene. Analysis revealed the sequence of this gene was conserved among. . .

DETD [0091] Alignment of nucleic acid sequences from E. ***chaffeensis*** p28 and Cowdria ruminantium map-1 using the Jotun-Hein aligorithm produced a consensus sequence with regions of high homology (>90%). These homologous regions (nucleotides 313-332 and 823-843 of C. ruminantium map-1; 307-326 and 814-834 of E. ***chaffeensis*** p28) were targeted as primer annealing sites for PCR amplification. PCR amplification of the E. ***canis*** p28-7 gene was accomplished with primers 793' (5-GCAGGAGCTGTTGGTTACTC-3') (SEQ ID NO. 16) and 1330 (5'-CCTTCCTCCAAGTTCTATGCC-3') (SEQ ID NO. 17), resulting in a 518-bp PCR product. E. ***canis*** DNA was amplified with primers 793 and 1330 with a thermal cycling profile of 95.degree. C. for 2 min, and. . . followed by a 72.degree. C. extension for 10 min and 4.degree. C. hold. The nucleic acid sequence of the E. ***canis*** PCR product was obtained by sequencing the product directly with primers 793 and 1330.

DETD . . . . frame encoding a protein of 170 amino acids, and alignment of the 518-bp sequence obtained from PCR amplification of E. ***canis*** with the DNA sequence of E. ***chaffeensis*** p28 gene revealed a similarity greater than 70%, indicating that the genes were homologous.

DETD . . . PCR product amplified with these primers was sequenced directly with the same primers. The complete DNA sequence for the E. ***canis*** p.sup.28-7 gene (SEQ ID NO. 1) is shown in FIG. 1. The p28-7 PCR fragment amplified with these primers contained. . . amino acids from the multiple cloning site and 5' non-coding primer region at the N-terminus. Convalescent-phase antiserum from an E. ***canis*** infected dog recognized the expressed recombinant fusion protein, but did not react with the thioredoxin control (FIG. 2).

DETD [0096] Sequence Homology of E. ***canis*** p28-7 Gene

DETD [0097] The nucleic acid sequence of E. ***canis*** p28-7 (834-bp) and the E. ***chaffeensis** omp-1 family of genes including signal sequences (p28-7, omp-1A, B, C, D, E, and F) were aligned using the Clustal method to examine homology between these genes (alignment not

shown). Nucleic acid homology was equally conserved (68.9%) between E. ***canis*** p28-7, E. ***chaffeensis*** p28 and omp-1F. Other putative outer membrane protein genes in the E. ***chaffeensis*** omp-1 family, omp-1D (68.2%), omp-1E (66.7%), omp-1C (64.1%), Cowdria ruminantium map-1 (61.8%), E. ***canis*** 28-kDa protein 1 gene (60%) and 28-kDa protein 2 gene (partial) (59.5%) were also homologous to p28-7. E. ***chaffeensis*** omp-1B had the least nucleic acid homology (45.1%) with E. ***canis*** p28-7.

DETD [0098] Alignment of the predicted amino acid sequences of E. ***canis*** P28-7 (SEQ ID NO. 2) and E. ***chaffeensis*** P28 revealed amino acid substitutions resulting in four variable regions (VR). Substitutions or deletions in the amino acid sequence and the locations of variable regions of E ***canis*** P28-7 and the E. ***chaffeensis*** OMP-1 family were identified (FIG. 3). Amino acid comparison including the signal peptide revealed that E. ***canis*** P28-7 shared the most homology with OMP-1F (68%) of the E. ***chaffeensis*** OMP-1 family, followed by E. ***chaffeensis*** P28 (65.5%), OMP-1E (65.1%), OMP-1D (62.9%), OMP-1C (62.9%), Cowdria ruminantium MAP-1 (59.4%), E. ***canis*** 28-kDa protein 1 (55.6%) and 28-kDa protein 2 (partial) (53.6%), and OMP-1B (43.2%). The phylogenetic relationships based on amino acid sequences show that E. ***canis*** P28-7 and C. ruminantium MAP-1, E. ***chaffeensis*** OMP-1 proteins, and E. ***canis*** 28-kDa proteins 1 and 2 (partial) are related (FIG. 4).

DETD [0099] Predicted Surface Probability and Immunoreactivity of E. ***canis*** P28-7

DETD [0100] Analysis of E. ***canis*** P28-7 using hydropathy and hydrophilicity profiles predicted surface-exposed regions on P28-7 (FIG. 6). Eight major surface-exposed regions consisting of 3 to 9 amino acids were identified on E. ***canis*** P28-7 and were similar to the profile of surface-exposed regions on E. ***chaffeensis*** P28 (FIG. 6). Five of the larger surface-exposed regions on E. ***canis*** P28-7 were located in the N-terminal region of the protein. Surface-exposed hydrophilic regions were found in all four of the variable regions of E. ***canis*** P28-7. Ten T-cell motifs were predicted in the P28-7 using the Rothbard-Taylor aligorithm (Rothbard and Taylor, 1988), and high antigenicity of the E. ***canis*** P28-7 was predicted by the Jameson-Wolf antigenicity aligorithm (FIG. 6) (Jameson and Wolf, 1988). Similarities in antigenicity and T-cell motifs were observed between E. ***canis*** P28-7 and E. ***chaffeensis*** P28.

DETD [0101] Detection of Homologous Genomic Copies of E. ***canis*** p28-7 Gene

DETD [0102] Genomic Southern blot analysis of E. ***canis*** DNA completely digested independently with restriction enzymes BanII, EcoRV,

HaeII, KpnI, SpeI, which do not have restriction endonuclease sites in. . . nucleotides 34, 43 and 656, revealed the presence of at least three homologous p28-7 gene copies (FIG. 5). Although E. ***canis*** p.sup.28-7 has internal Ase I internal restriction sites, the DIG-labeled probe used in the hybridization experiment targeted a region of. . .

DETD [0103] PCR Amplification of E. ***canis*** ECa28SA2 (p28-5), ECa28SA3 (p28-6) Genes and Identification of the Multiple Gene Locus

DETD [0108] Nucleic and Amino Acid Homology of E. ***canis*** p28-4, p28-5, p28-6, p28-7 and p28-8 Proteins

DETD [0109] The nucleic and amino acid sequences of all five E. ***canis*** 28-kDa protein genes were aligned using the Clustal method to examine the homology between these genes. The nucleic acid homology. . . 58 to 75% and a similar amino acid homology of ranging from 67 to 72% was observed between the E. ***canis*** 28-kDa protein gene members (FIG. 9).

DETD . . . protein genes were analyzed for promoter sequences by comparison with consensus Escherichia coli promoter regions and a promoter from E. ***chaffeensis*** (Yu et al., 1997; McClure, 1985). Putative promoter sequences including RBS, -10 and -35 regions were identified in 4 intergenic. . .

DETD [0113] The amino acid sequence analysis revealed that entire E. ***canis*** p28-7 has a deduced molecular mass of 30.5-kDa and the entire p28-6 has a deduced molecular mass of 30.7-kDa. Both. . . N-terminal signal peptide of 23 amino acids (MNCKKILITTALMSLMYYAPSIS, SEQ ID No. 27), which is similar to that predicted for E. ***chaffeensis*** P28 (MNYKKILITSALISLISSLPGV SFS, SEQ ID NO. 28), and the OMP-1 protein family (Yu et al., 1999a; Ohashi et al., 1998b).

DETD . . . cleavage site at amino acid position 25 (MNCKKILITTALISLMYSIPSISSFS, SEQ ID NO. 29) identical to the predicted cleavage site of E. ***chaffeensis*** P28 (SFS) was also present, and would result in a mature p28-7 with a predicted molecular mass of 27.7-kDa. Signal. . .

DETD [0115] Proteins of similar molecular mass have been identified and cloned from multiple rickettsial agents including E. ***canis*** , E. ***chaffeensis*** , and C. ruminantium (Reddy et al., 1998; Jongejan et al., 1993; Ohashi et al., 1998). A single locus in ***Ehrlichia*** ***chaffeensis*** with 6 homologous p28 genes, and-2 loci in E. ***canis*** , each containing some homologous 28-kDa protein genes have been previously described.

DETD [0116] The present invention demonstrated the cloning, expression and characterization of genes encoding mature 28-kDa proteins of E. ***canis*** that are homologous to the omp-1 multiple gene family of E. ***chaffeensis*** and the C. ruminantium map-1 gene. Two new 28-kDa protein genes were identidfied, p28-7 and p28-6. Another E.

canis 28-kDa protein gene, p28-5, partially sequenced previously (Reddy et al., 1998), was sequenced completely in the present invention. Also disclosed is the identification and characterization of a single locus in E. ***canis*** containing five E. ***canis*** 28-kDa protein genes (p28-4, p28-5, p28-6, p28-7 and p28-8).

DETD [0117] The E. ***canis*** 28-kDa proteins are homologous to E. ***chaffeensis*** OMP-1 family and the MAP-1 protein of C. rumanintium. The most homologous E. ***canis*** 28-kDa proteins (p28-6, p28-7 and p28-8) are sequentially arranged in the locus. Homology of these proteins ranged from 67.5% to 72.3%. Divergence among these 28-kDa proteins was 27.3% to 38.6%. E ***canis*** 28-kDa proteins p28-4 and p28-5 were the least homologous with homology ranging from 50.9% to 59.4% and divergence of 53.3. . . these regions are surface exposed and subject to selective pressure by the immune system. Conservation of p28-7 among seven E. ***canis*** isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be clonal in North America. Conversely, significant diversity of p28 among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a).

DETD [0118] All of the E. ***canis*** 28-kDa proteins appear to be post translationally processed from a 30-kD protein to a mature 28-kD protein. Recently, a signal sequence was identified on E. ***chaffeensis*** P28 (Yu et al., 1999a), and N-terminal amino acid sequencing has verified that the protein is post-translationally processed resulting in. . . OMP-1F and OMP-1E have also been proposed as leader signal peptides (Ohashi et al., 1998). Signal sequences identified on E. ***chaffeensis*** OMP-1F, OMP-1E and P28 are homologous to the leader sequence of E. ***canis*** 28-kDa protein. Promoter sequences for the p28 genes have not been determined experimentally, but putative promoter regions were identified by comparison with consensus sequences of the RBS, -10 and -35 promoter regions of E. coli and other ***ehrlichiae*** (Yu et al., 1997; McClure, 1985). Such promoter sequences would allow each gene to potentially be transcribed and translated, suggesting. . .

DETD [0119] The E. ***canis*** 28-kda protein genes were found to exhibit nucleic acid and amino acid sequence homology with the E. ***chaffeensis*** omp-1 gene family and C. ruminantium map-1 gene. Previous studies have identified a 30-kDa protein of E. ***canis*** that reacts with convalescent phase antisera against E. ***chaffeensis*** , but was believed to be antigenically distinct (Rikihisa et al., 1994). Findings based on comparison of amino acid substitutions in four variable regions of E. ***canis*** 28-kDa proteins support this possibility. Together these findings also suggest that the amino acids responsible for the antigenic differences between E. ***canis*** and E. ***chaffeensis*** P28 are located in these

variable regions and are readily accessible to the immune system.

DETD . . . It was reported that immunoreactive peptides were located in the variable regions of the 28-kDa proteins of C. ruminantium, E. ***chaffeensis*** and E. ***canis*** (Reddy et al., 1998). Analysis of E. ***canis*** and E. ***chaffeensis*** P28 revealed that all of the variable regions have predicted surface-exposed amino acids. A study in dogs demonstrated lack of cross protection between E. ***canis*** and E. ***chaffeensis*** (Dawson and Ewing, 1992). This observation may be related to antigenic differences in the variable regions of P28 as well as in other immunologically important antigens of these ***ehrlichial*** species. Another study found that convalescent phase human antisera from E. ***chaffeensis*** -infected patients recognized 29/28-kDa protein(s) of E. ***chaffeensis*** and also reacted with homologous proteins of E. ***canis*** (Chen et al., 1997). Homologous and crossreactive epitopes on the E. ***canis*** 28-kDa protein and E. ***chaffeensis*** P28 appear to be recognized by the immune system.

DETD [0121] E. ***canis*** 28-kDa proteins may be important immunoprotective antigens. Several reports have demonstrated that the 30-kDa antigen of E. ***canis*** exhibits strong immunoreactivity (Rikihisa et al., 1994; Rikihisa et al., 1992). Antibodies in convalescent phase antisera from humans and dogs have consistently reacted with proteins in this size range from E. ***chaffeensis*** and E. ***canis*** , suggesting that they may be important immunoprotective antigens (Rikihisa et al., 1994; Chen et al., 1994; Chen et al., 1997). In addition, antibodies to 30, 24 and 21-kDa proteins developed early in the immune response to E. ***canis*** (Rikihisa et al., 1994; Rikihisa et al., 1992), suggesting that these proteins may be especially important in the immune responses. . . Recently, a family of homologous genes encoding outer membrane proteins with molecular masses of 28-kDa have been identified in E. ***chaffeensis*** , and mice immunized with recombinant E. ***chaffeensis*** P28 appeared to have developed immunity against homologous challenge (Ohashi et al., 1998). The P28 of E. ***chaffeensis*** has been demonstrated to be present in the outer membrane, and immunoelectron microscopy has localized the P28 on the surface. . . that it may serve as an adhesin (Ohashi et al., 1998). It is likely that the 28-kDa proteins of E. ***canis*** identified in this study have the same location and possibly serve a similar function.

DETD [0122] Comparison of p28-7 from different strains of E. ***canis*** revealed that the gene is apparently completely conserved. Studies involving E. ***chaffeensis*** have demonstrated immunologic and molecular evidence of diversity. Patients infected with E. ***chaffeensis*** have variable immunoreactivity to the 29/28-kDa

proteins, suggesting that there is antigenic diversity (Chen et al., 1997). Recently molecular evidence has been generated to support antigenic diversity in the p28 gene from E. ***chaffeensis*** (Yu et al., 1999a). A comparison of five E. ***chaffeensis*** isolates revealed that two isolates (Sapulpa and St. Vincent) were 100% identical, but three others (Arkansas, Jax, 91HE17) were divergent by as much as 13.4% at the amino acid level. The conservation of E. ***canis*** p28-7 suggests that E. ***canis*** strains found in the United States may b e genetically identical, and thus E. ***canis*** 28-kDa protein is an attractive vaccine candidate for canine ***ehrlichiosis*** in the United States. Further analysis of E. ***canis*** isolates outside the United States may provide information regarding the origin and evolution of E. ***canis*** . Conservation of the 28-kDa protein makes it an important potential candidate for reliable serodiagnosis of canine ***ehrlichiosis*** .

DETD [0123] The role of multiple homologous genes is not known at this point; however, persistence of E. ***canis*** infections in dogs could conceivably be related to antigenic variation due to variable expression of homologous 28-kDa protein genes, thus enabling E ***canis*** to evade immune surveillance. Variation of msp-3 genes in A. marginale is partially responsible for variation in the MSP-3 protein, resulting in persistent infections (Alleman et al., 1997). Studies to examine 28-kDa protein gene expression by E. ***canis*** in acutely and chronically infected dogs would provide insight into the role of the 28-kDa protein gene family in persistence. . .

DETD [0124] Identification of E. ***canis*** p28-1, p28-2, P28-3 and p28-9 Genes

DETD [0126] The nucleic acid and amino acid sequences of the E. ***canis*** p28 genes were aligned using the Clustal method to examine the homology between these genes. Homology of these proteins ranged from 67.5% to 75%, and divergence among these P28 proteins was 26.9% to 38%. E. ***canis*** P28 proteins P28-1, P28-2, and P28-9 were the least homologous with the other p28 genes ranging from 37% to 49%. . . nucleic acid homology of the nine p28 genes ranged from 28 to 72%. The phylogenetic relationships based on the E. ***canis*** p28 amino acid sequences are shown in FIG. 12.

DETD . . . accession numbers. The GenBank accession numbers for the nucleic acid and amino acid sequences for the complete nine gene E. ***canis*** (Jake strain) p28 gene locus is AF082744. This accession number was originally assigned to p28-7, but has been updated with. . . p28 locus, which includes p28-7. GenBank accession numbers for nucleic acid and amino acid sequences of p28-7 in other E. ***canis*** isolates described in this study are: Louisiana, AF082745; Oklahoma, AF082746; Demon, AF082747; DJ, AF082748; Fuzzy, AF082749; Florida, AF082750.

DETD [0128] Multiple bands in the 28-kilodalton range have been observed by immunoblots of convalescent sera from E. ***canis*** infected dogs (Rikihisa et al., 1994), and expression of multiple p28 proteins could be an explanation for this observation. Southern. . .

DETD [0129] In this study a single gene locus containing nine tandemly arranged E. ***canis*** p28 genes encoding homologous, but nonidentical, p28 genes was identified. The nine gene locus included four new p28 genes (FIGS.. . .

DETD [0130] The P28s of E. ***canis*** were found to be as closely related to 28-kilodalton proteins of other species such as E. ***chaffeensis*** as they are to themselves (McBride et al., 2000). Differences among the proteins are found primarily in several major hypervariable. . .

DETD [0131] Conservation of an E. ***canis*** p28 gene (p28-7) among seven geographically different isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be highly conserved in North America. Similarly, the 120-kDa glycoprotein of E. ***canis*** is also conserved among isolates in the United States (Yu et al., 1997). In contrast, both the 120-kDa and the 28-kDa protein genes of E. ***chaffeensis*** are divergent among isolates (Yu et al., 1999a; Chen et al., 1997). The diversity of the 28-kDa protein gene of E. ***chaffeensis*** appeared to result from point mutations in the hypervariable regions perhaps due to selective immune pressure (Yu et al., 1999a). These data suggest that E. ***canis*** may have been introduced into North America relatively recently, and this may account for the conservation that was observed among isolates. The conservation of p28 genes in E. ***canis*** isolates may provide an opportunity to develop vaccine and serodiagnostic antigens that are particularly effective for disease prevention and serodiagnosis.. . .

DETD [0161] Troy G. C., et al., (1990) Canine ***ehrlichiosis*** . In Infectious diseases of the dog and cat . Green C. E. (ed). Philidelphia: W.B. Sauders Co.

DETD . . . NUMBER OF SEQ ID NOS: 46

<210> SEQ ID NO 1
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<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-7

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atctctaatg ttttatacct aatatatata ttctggcttg tatctacttt 100
. . . tagacccaag 1600

tacaatg 1607

<210> SEQ ID NO 2
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-7 protein

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275

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<400> SEQUENCE: 3

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<400> SEQUENCE: 4

Met Asn Cys Lys. . . Asn Phe
275 280

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<400> SEQUENCE: 6

Met Asn Cys Lys. . . Asn Phe
275 280

<210> SEQ ID NO 7
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<400> SEQUENCE: 7

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<210> SEQ ID NO 8
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<213> ORGANISM: ***Ehrlichia*** ***canis***
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<400> SEQUENCE: 8

Met Lys Tyr Lys. . . 280 285

Phe Phe

<210> SEQ ID NO 9
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** P28

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275 280

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275 280

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<220> FEATURE:
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275 280

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280 285

Leu

<210> SEQ ID NO 13
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<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***chaffeensis*** OMP-1E

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275

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also nucleotides 307-326 of E. ***chaffeensis*** P28
<223> OTHER INFORMATION: forward primer 793 for PCR

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<210>. . . sequence
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<222> LOCATION: nucleotides 823-843 of C. ruminantium MAP-1,
also nucleotides 814-834 of E. ***chaffeensis*** P28
<223> OTHER INFORMATION: reverse primer 1330 for PCR

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. . . artificial sequence
<220> FEATURE:
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genes in E. ***canis***

<400> SEQUENCE: 19

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genes in E. ***canis***

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<223> OTHER INFORMATION: primer 394 for PCR

<400> SEQUENCE: 21

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<210>. . . LENGTH: 24
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<213> ORGANISM: artificial sequence
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<221> NAME/KEY: primer_bind
<222> LOCATION: nucleotides 710-687 of E. ***canis*** p28-7
<223> OTHER INFORMATION: primer 394C for PCR

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<210>. . . artificial sequence
<220> FEATURE:
<221> NAME/KEY: primer_bind
<223> OTHER INFORMATION: primer 793C which anneals to a region with E. ***canis*** p28-7, used to amplify the intergenic region between gene p28-6 and p28-7

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ctaggattag gttatagtat aagtt 25

<210> SEQ ID NO 27
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<213> ORGANISM: ***Ehrlichia*** ***canis***

<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<223> OTHER INFORMATION: a predicted N-terminal signal peptide of p28-7 and p28-6

<400>. . . Ser Ile Ser
20

<210> SEQ ID NO 28
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: ***Ehrlichia***. ***chaffeensis***
<220> FEATURE:
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<400> SEQUENCE: 28

Met Asn Tyr Lys Lys Ile Leu Ile Thr Ser Ala Leu Ile Ser. . . Phe Ser
20 25

<210> SEQ ID NO 29
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<223> OTHER INFORMATION: amino acid sequence of putative cleavage site of p28-7

<400> SEQUENCE: 29
. . . Phe Ser
20 25

<210> SEQ ID NO 30
<211> LENGTH: 299
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 1 (28NC1)

<400> SEQUENCE: 30

. . . tactactgtt aatttacttt cactgtttct ggtgtaaat 299

<210> SEQ ID NO 31
<211> LENGTH: 345
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 2 (28NC2)

<400> SEQUENCE: 31
. . . tattgttaat ttattttcac tattttaggt gtaat 345

<210> SEQ ID NO 32
<211> LENGTH: 345
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 3 (28NC3)

<400> SEQUENCE: 32
. . . tattgttaat ttattttcac tattttaggt gtaat 345

<210> SEQ ID NO 33
<211> LENGTH: 355
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of intergenic noncoding region 4 (28NC4)

<400> SEQUENCE: 33
. . .

aatccatcat ttctcattac agtgtg 26

<210> SEQ ID NO 39
<211> LENGTH: 879
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***

<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-1

<400> SEQUENCE: 39

atgaataata aactcaaatt tactataata aacacagtat tagtatgctt 50

attgtcatta cctaatatat cttcctcaaa ggccataaac aataacgcta 100
. . .

gaggtgaaat tggtgcaaga ttgacattt 879

<210> SEQ ID NO 40
<211> LENGTH: 293
<212> TYPE: PRT
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-1
protein

<400> SEQUENCE: 40

Met Asn Asn Lys Leu Lys Phe Thr Ile Ile Asn Thr Val. . .
DETD . . . Thr Phe
290 293

<210> SEQ ID NO 41
<211> LENGTH: 840
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-2

<400> SEQUENCE: 41

atgaattata agaaaattct agtaagaagc gcgttaatct cattaatgtc 50

aatcttacca tatcagtctt ttgcagatcc tgtaggttca agaactaatg 100
. . .
tggatacttt ggcggagaaa ttggaatgag gttcaccttc 840

<210> SEQ ID NO 42

<211> LENGTH: 280
<212> TYPE: PRT
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-2 protein

<400> SEQUENCE: 42

Met Asn Tyr Lys Lys Ile Leu Val Arg Ser Ala Leu Ile. . . Thr Phe
275 280

<210> SEQ ID NO 43
<211> LENGTH: 828
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-3

<400> SEQUENCE: 43

atgaactgta aaaaaattct tataacaact acattggtat cactaacaat 50

tcttttacct ggcatatctt tctccaaacc aatacatgaa aacaatacta 100
. . .

gttagaattt gggtacaggg tcagtttt 828

<210> SEQ ID NO 44
<211> LENGTH: 276
<212> TYPE: PRT
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-3 protein

<400> SEQUENCE: 44

Met Asn Cys Lys Lys Ile Leu Ile Thr Thr Thr Leu Val. . . Val Ser Phe
275

<210> SEQ ID NO 45

<211> LENGTH: 813
<212> TYPE: DNA
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence of E. ***canis*** p28-9

<400> SEQUENCE: 45

atgaattaca aaagatttgt tgtaggtgtt acgctgagta catttgtttt 50

tttcttatct gatggtgctt tttctgatgc aaatttttct gaagggagga 100
. . . 800

tagatttgcg cta 813

<210> SEQ ID NO 46
<211> LENGTH: 271
<212> TYPE: PRT
<213> ORGANISM: ***Ehrlichia*** ***canis***
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of E. ***canis*** p28-9 protein

<400> SEQUENCE: 46

Met Asn Tyr Lys Arg Phe Val Val Gly Val Thr Leu Ser. . .

CLM What is claimed is:

1. An isolated DNA sequence encoding a 30-kilodalton protein of ***Ehrlichia*** ***canis*** , wherein said protein is immunoreactive with anti- ***Ehrlichia*** ***canis*** serum.

6. The DNA sequence of claim 1, wherein said DNA is contained in a single locus of ***Ehrlichia*** ***canis*** .

8. The DNA sequence of claim 7, wherein said locus contains genes encoding homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** .

9. The DNA sequence of claim 8, wherein said homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** are selected from the group consisting of p28-1, p28-2, p.sup.28-3, p28-4, p28-5, p28-6, p28-7, p28-8 and p28-9.

17. A method of inhibiting ***Ehrlichia*** ***canis*** infection

in a subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit ***Ehrlichia*** ***canis*** infection.

L7 ANSWER 15 OF 30 USPATFULL
AN 2002:116392 USPATFULL
TI Homologous 28-kilodalton immunodominant protein genes of ***Ehrlicha*** ***canis*** and uses thereof
IN Walker, David H., Galveston, TX, United States
Yu, Xue-Jie, Houston, TX, United States
McBride, Jere W., Galveston, TX, United States
PA Research Development Foundation, Carson City, NV, United States (U.S. corporation)
PI US 6392023 B1 20020521
AI US 2000-660587 20000912 (9)
RLI Continuation-in-part of Ser. No. US 1999-261358, filed on 3 Mar 1999
Continuation-in-part of Ser. No. US 1958-201458, filed on 30 Nov 1958
DT Utility
FS GRANTED
EXNAM Primary Examiner: Low, Christopher S. F.; Assistant Examiner: Schnizer, Holly
LREP Adler, Benjamin Aaron
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 17 Drawing Figure(s); 20 Drawing Page(s)
LN.CNT 1266
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.
TI Homologous 28-kilodalton immunodominant protein genes of ***Ehrlicha*** ***canis*** and uses thereof
AB . . . of homologous immunoreactive 28-kDa protein genes, p28-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of ***Ehrlichia*** ***canis*** . Further disclosed is a multigene

locus encoding all nine homologous 28-kDa protein genes of ***Ehrlichia*** ***canis*** . Recombinant ***Ehrlichia*** ***canis*** 28-kDa proteins react with convalescent phase antiserum from an E. ***canis*** -infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and. . .

SUMM . . . of molecular biology. More specifically, the present invention relates to molecular cloning and characterization of homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , a multigene locus encoding the 28-kDa homologous proteins of ***Ehrlichia*** ***canis*** and uses thereof.

SUMM Canine ***ehrlichiosis*** , also known as canine tropical pancytopenia, is a tick-borne rickettsial disease of dogs first described in Africa in 1935 and. . .

SUMM The etiologic agent of canine ***ehrlichiosis*** is ***Ehrlichia*** ***canis*** , a small, gram-negative, obligate intracellular bacterium which exhibits tropism for mononuclear phagocytes (Nyindo et al. 197 1) and is transmitted by the brown dog tick, Rhipicephalus sanguines (Groves et al., 1975). The progression of canine ***ehrlichiosis*** occurs in three phases, acute, subclinical and chronic. The acute phase is characterized by fever, anorexia, depression, lymphadenopathy and mild. . .

SUMM . . . persistent infections in the host. Although disease pathogenesis is poorly understood, multigene families described in members of the related genera ***Ehrlichia*** , Anaplasma, and Cowdria may be involved in variation of major surface antigen expression thereby evading immune surveillance. Anaplasma marginale, an organism closely related to E. ***canis*** , exhibits variation of major surface protein 3 (msp-3) genes resulting in antigenic polymorphism among strains (Alleman et al., 1997).

SUMM Molecular taxonomic analysis based on the 16S rRNA gene has determined that E. ***canis*** and E. ***chaffeensis*** , the etiologic agent of human monocytic ***ehrlichiosis*** (HME), are closely related (Anderson et al., 1991; Anderson et al., 1992; Dawson et al., 1991; Chen et al., 1994). Considerable cross reactivity of the 64, 47, 40, 30, 29 and 23-kDa antigens between E. ***canis*** and E. ***chaffeensis*** has been reported (Chen et al., 1994; Chen et al., 1997; Rikihisa et al., 1994; Rikihisa et al., 1992). Analysis. . . with human and canine convalescent phase sera by immunoblot has resulted in the identification of numerous immunodominant proteins of E. ***canis*** , including a 30-kDa protein (Chen et al., 1997). In addition, a 30-kDa protein of E. ***canis*** has been described as a major immunodominant antigen recognized early in the immune response that is antigenically distinct from the 30-kDa protein of E. ***chaffeensis*** (Rikihisa et al., 1992; Rikihisa et al., 1994).

Other immunodominant proteins of E. ***canis*** with molecular masses ranging from 20 to 30-kDa have also been identified (Brouqui et al., 1992; Nyindo et al., 1991;. . .

SUMM Homologous 28-32kDa immunodominant proteins encoded by multigene families have been reported in related organisms including, E. ***chaffeensis*** and Cowdria ruminantium (Sulsona et al., 1999; Ohashi et al., 1998a; Reddy et al., 1 998). Recently, characterization of a 21 member multigene family encoding proteins of 23 to 28-kDa has been described in E. ***chaffeensis*** (Yu et al., 2000). The E. chaffeensis28-kDa outer membrane proteins are surface exposed, and contain three major hypervariable regions (Ohashi et al., 1998a). The recombinant E. ***chaffeensis*** P28 appeared to provide protection against homologous challenge infection in mice, and antisera produced against the recombinant protein cross reacted with a 30-kDa protein of E. ***canis*** (Ohashi et al., 1998a). Diversity in the p28 gene among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a), and studies using monoclonal antibodies have further demonstrated diversity in the expressed P28 proteins (Yu et al., 1993). Conversely, complete conservation of a p28 genes in geographically different isolates of E. ***canis*** has been reported and suggests that E. ***canis*** may be conserved in North America (McBride et al., 1999, 2000).

SUMM The prior art is deficient in the lack of cloning and characterization of new homologous 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** and a single multigene locus containing the homologous 28-kDa protein genes. Further, The prior art is deficient in the lack of recombinant proteins of such immunoreactive genes of ***Ehrlichia*** ***canis*** . The present invention fulfills this long-standing heed and desire in the art.

SUMM . . . of the present invention describe the molecular cloning, sequencing, characterization, and expression of homologous mature 28-kDa immunoreactive protein genes of ***Ehrlichia*** ***canis*** (designated p28-1, -2, -3, -5, -6, -7, -9), and the identification of a single locus (10,677-bp) containing nine 28-kDa protein genes of ***Ehrlichia*** ***canis*** (p28-1 to p28-9). Eight of the p28 genes were located on one DNA strand, and one p28 gene was found. . .

SUMM In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . . contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

SUMM . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive

protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

SUMM The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject prior to exposure or suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, e.g., the stimulation of the subject's humoral or cellular immune. . .

DRWD . . . arrow) and 16-kDa thioredoxin control (Lane 2, arrow), and corresponding immunoblot of recombinant p28-7-thioredoxin fusion protein recognized by covalescent-phase E. ***canis*** canine antiserum (Lane 3). Thiroredoxin control was not detected by E. ***canis*** antiserum (not shown).

DRWD . . . ID NO. 2), p28-5 protein (ECa28SA2, partial sequence, SEQ ID NO. 7), p28-4 protein (ECa28SA1, SEQ ID NO. 8), E. ***chaffeensis*** P28 (SEQ ID NO. 9), E. ***chaffeensis*** OMP-1 family (SEQ ID NOs: 10-14) and C. rumanintium MAP-1 protein (SEQ ID NO. 15). The p28-7 amino acid sequence. . .

DRWD FIG. 4 shows phylogenetic relatedness of E. ***canis*** p28-7 (ECa28-1), p28-5 (ECa28SA2, partial sequence), p28-4 (ECa28SA1), members of the E. ***chaffeensis*** omp-1 multiple gene family, and C. rumanintium map-1 protein from deduced amino acid sequences utilizing unbalanced tree construction. The length. . .

DRWD FIG. 5 shows Southern blot analysis of E. ***canis*** genomic DNA completely digested with six individual restriction enzymes and hybridized with a p28-7 DIG-labeled probe (Lanes 2-7); DIG-labeled molecular. . .

DRWD FIG. 6 shows comparison of predicted protein characteristics of E. ***canis*** p28-7 (ECa28-1, Jake strain) and E. ***chaffeensis*** P28 (Arkansas strain). Surface probability predicts the surface residues by using a window of hexapeptide. A surface residue is any. . . .

DRWD FIG. 8 shows schematic of the E. ***canis*** 28-kDa protein gene locus (5.592-Kb, containing five genes) indicating genomic orientation and intergenic noncoding regions (28NC1-4). The 28-kDa protein genes. .

DRWD FIG. 9 shows phylogenetic relatedness of the E. ***canis*** 28-kDa protein gene p28-4 (ECa28SA1), p28-5 (ECa28SA2), p28-6 (ECa28SA3), p28-7 (ECa28-1) and p28-8 (ECa28-2) based on amino acid sequences utilizing.

DRWD FIG. 10 shows alignment of E. ***canis*** 28-kDa protein gene intergenic noncoding nucleic acid sequences (SEQ ID Nos. 30-33). Nucleic acids not shown, denoted with a dot. . .

DRWD FIG. 11 shows schematic representation of the nine gene E. ***canis*** p28 locus (10,677-bp) indicating genomic orientation and intergenic noncoding regions. The p28 genes (p28-1, 2, 3, 9) (unshaded) were identified in Example 8. Shaded p28 genes have been identified previously and designated as follows: p28-4, ***p30a*** (Ohashi et al., 1998b) and ORF1 (Reddy et al., 1998); p28-5 and p28-6, (McBride, et.al., 2000); p28-7, p28 (McBride et al., 1999) and ***p30*** (Ohashi et al., 1998b); and p28-8, ***p30*** -1 (Ohashi et al., 1998b).

DRWD FIG. 12 shows phylogenetic relationships of E. ***canis*** P28-1 to P28-9 based on the amino acid sequences. The length of each pair of branches represents the distance between. . .

DRWD . . . 13 shows nucleic acid sequence (SEQ ID No. 39) and deduced amino acid sequence (SEQ ID No. 40) of E. ***canis*** p28-1 gene.

DRWD . . . shows nucleic acid sequence (SEQ ID No. 4 1) and deduced amino acid sequence (SEQ ID No. 42) of E. ***canis*** p28-2 gene.

DRWD . . . 15 shows nucleic acid sequence (SEQ ID No. 43) and deduced amino acid sequence (SEQ ID No. 44) of E. ***canis*** p28-3 gene.

DRWD . . . 16 shows nucleic acid sequence (SEQ ID No. 45) and deduced amino acid sequence (SEQ ID No. 46) of E. ***canis*** p28-9 gene.

DETD The present invention describes cloning, sequencing and expression of homologous genes encoding a 30-kilodalton (kDa) protein of ***Ehrlichia*** ***canis*** . A comparative molecular analysis of homologous genes among seven E. ***canis*** isolates and the E. ***chaffeensis*** omp-1 multigene family was also performed. Several new 28-kDa protein genes are identified as follows:

DETD Using PCR to amplify 28-kDa protein genes of E. ***canis*** , a previously unsequenced region of p28-5 (Eca28SA2) was completed. Sequence analysis of p28-5 revealed an 849-bp open reading frame encoding. . .

DETD . . . of tandemly arranged p28 genes were sequenced, and p28-1, -2, -3, and -9 were identified. Consequently, a nine gene E. ***canis*** p28 locus spanning 10, 677 bp was identified in the present invention.

DETD The present invention is directed to, inter alia, homologous 28-kDa protein genes in ***Ehrlichia*** ***canis*** , p28-1, -2, -3, -6, -7, and p28-9, and a complete sequence of previously partially sequenced p28-5. Also disclosed is a multigene locus encoding nine homologous 28-kDa outer membrane proteins of ***Ehrlichia*** ***canis*** . Eight of the p28 genes were located on one DNA strand, and one p28 gene was found on the complementary. . .

DETD The invention includes a substantially pure DNA encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably. . .

DETD . . . listed in SEQ ID No 1, 3, 5, 39, 41, 43, or 45 which encodes a

28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** .

DETD . . . comprises a vector comprising a DNA sequence coding for a which encodes a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and said vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication;. . .

DETD . . . or viral nucleic acid. Vectors may be used to amplify and/or express nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to. . .

DETD . . . such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention can be used to transform a host using any of the techniques commonly known to those. . . Especially preferred is the use of a vector containing coding sequences for a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** of the present invention for purposes of prokaryote transformation.

DETD . . . "recombinant" cell is intended to refer to a cell into which a recombinant gene, such as a gene encoding an ***Ehrlichia*** ***canis*** antigen has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced. . .

DETD The present invention is also drawn to substantially pure 28-30 kDa immunoreactive proteins of E. ***canis*** comprise of amino acid sequences listed in, for example, SEQ ID No. 2, 4, 6, 40, 42, 44, or 46.

DETD . . . more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** ; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography such as immunoaffinity chromatography using an antibody specific for a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from. . .

DETD In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (SEQ ID No. 2, 4, 6, 40, 42, 44, or 46). As used herein, "fragment," as applied to a polypeptide,. . . 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the 28-kDa immunoreactive

protein of ***Ehrlichia*** ***canis*** can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , by recombinant DNA techniques using an expression vector that encodes a defined fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** , or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** (e.g., binding to an antibody specific for 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis***) can be assessed by methods described herein.

DETD Purified 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** or antigenic fragments of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay). . .

DETD Included in this invention are polyclonal antisera generated by using 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** or a fragment of 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant ***Ehrlichia*** ***canis*** cDNA clones, and to distinguish them from known cDNA clones.

DETD In one embodiment of the present invention, there are provided DNA sequences encoding a 30-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** . Preferably, the protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2, 4, 6,. . .

contained in a single multigene locus, which has the size of 10,677 bp and encodes nine homologous 28-kDa proteins of ***Ehrlichia*** ***canis*** .

DETD . . . embodiment of the present invention, there is provided an expression vector comprising a gene encoding a 28-kDa immunoreactive protein of ***Ehrlichia*** ***canis*** and capable of expressing the gene when the vector is introduced into a cell.

DETD The invention may also be described in certain embodiments as a method of inhibiting ***Ehrlichia*** ***canis*** infection in a subject comprising the steps of: identifying a subject suspected of being exposed to or infected with ***Ehrlichia*** ***canis*** ; and administering a composition comprising a 28-kDa antigen of ***Ehrlichia*** ***canis*** in an amount effective to inhibit an ***Ehrlichia*** ***canis*** infection. The inhibition may occur through any means such as, i.e. the stimulation of the subject's humoral or cellular immune. . .

DETD ***Ehrlichia*** and Purification ***Ehrlichia*** ***canis*** (Florida strain and isolates Demon, DJ, Jake, and Fuzzy) were provided by Dr. Edward Breitschwerdt, (College of Veterinary Medicine, North Carolina State University, Raleigh, N.C.). E. ***canis*** (Louisiana strain) was provided by Dr. Richard E. Corstvet (School of Veterinary Medicine, Louisiana State University, Baton Rouge, La.) and E. ***canis*** (Oklahoma strain) was provided by Dr. Jacqueline Dawson (Centers for Disease Control and Prevention, Atlanta, Ga.). Propagation of ***Ehrlichiae*** was performed in DH82 cells with DMEM supplemented with 10% bovine calf serum and 2 mM L-glutamine at 37.degree. C. The intracellular growth in DH82 cells was monitored by presence of E. ***canis*** morulae using general cytologic staining methods. Cells were harvested when 100% of the cells were infected with ***Ehrlichiae*** and were then pelleted in a centrifuge at 17,000.times.g for 20 min. Cell pellets were disrupted with a Braun-Sonic 2000 sonicator twice at 40W for 30 sec on ice. ***Ehrlichiae*** were purified as described previously (Weiss et al., 1975). The lysate was loaded onto discontinuous gradients of 42%-36%-30% renografin, and centrifuged at 80,000.times.g for 1 hr. Heavy and light bands containing ***ehrlichia*** were collected and washed with sucrose-phosphate-glutamate buffer (SPG, 218 mM sucrose, 3.8 mM KH.sub.2PO.sub.4, 7.2 mM K.sub.2HPO.sub.4, 4.9 mM glutamate,. . .

DETD Nucleic Acid Preparation ***Ehrlichia*** ***canis*** genomic DNA was prepared by resuspending the renografin-purified ***ehrlichia*** in 600 .mu.l of 10 mM Tris-HCl buffer (pH 7.5) with 1% sodium dodecyl sulfate (SDS, w/v) and 100 ng/ml. . .

DETD . . . using a Universal LCI GenomeWalker Kit (CLONTECH, Palo Alto, Calif.) according to the protocol supplied by the manufacturer. Genomic E. ***canis*** (Jake isolate) DNA was digested completely with five restriction enzymes (DraI, EcoRV, PvuII, ScaI, StuI) which produce blunt-ended DNA. An adapter (AP1) supplied in the kit was ligated to each end of E. ***canis*** DNA. The genomic libraries were used as templates to find the unknown DNA sequence of the p28-7 gene by PCR. .

DETD . . . with an ABI Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The entire p28-7 genes of seven E. ***canis*** isolates (four from North Carolina, and one each from Oklahoma, Florida, and Louisiana) were amplified by PCR with primers EC28OM-F. . .

DETD PCR Amplification, Cloning, Sequencing and Expression of E. ***canis*** ECa28-1 (p28-7) Gene

DETD Expression Vectors The entire E. ***canis*** p28-7 gene was PCR-amplified with primers-EC28OM-F and EC28OM-R and cloned into pCR2.1-TOPO TA cloning vector to obtain the desired set. . .

DETD Western Blot Analysis Recombinant E. ***canis*** p28-7 fusion

protein was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-15% Tris-HCl gradient gels (Bio-Rad, Hercules, Calif.) and transferred. . . N.H.) using a semi-dry transfer cell (Bio-Rad, Hercules, Calif.). The membrane was incubated with convalescent phase antisera from an E. ***canis*** -infected dog diluted 1:5000 for 1 hour, washed, and then incubated with an anti-canine IgG (H & L) alkaline phosphatase-conjugated affinity-purified. . .

DETD Southern Blot Analysis To determine if multiple genes homologous to the p28-7 gene were present in the E. ***canis*** genome, a genomic Southern blot analysis was performed using a standard procedure (Sambrook et al. 1989). E. ***canis*** genomic DNA digested completely with each of the restriction enzymes BanII, EcoRV, HaeII, KpnI and SpeI, which do not cut. . . digested probe (566-bp) was separated by agarose gel electrophoresis, gel-purified and then used for hybridization. The completely digested genomic E. ***canis*** DNA was electrophoresed and transferred to a nylon membrane (Boehringer Mannheim, Indianapolis, Ind.) and hybridized at 40.degree. C. for 16. .

DETD Sequence Analysis and Comparasion E. ***chaffeensis*** p28 and C. rumiantium map-1 DNA sequences were obtained from the National Center of Biotechnology Information (NCBI). Nucleotide and deduced. . .

DETD Sequence analysis of p28-7 from seven different strains of E. ***canis*** was performed with primers designed to amplify the entire gene. Analysis revealed the sequence of this gene was conserved among. . .

DETD Alignment of nucleic acid sequences from E. ***chaffeensis*** p28 and Cowdria ruminantium map-1 using the Jotun-Hein aligorithm produced a consensus sequence with regions of high homology (>90%). These homologous regions (nucleotides 313-332 and 823-843 of C. ruminantium map-1; 307-326 and 814-834 of E. ***chaffeensis*** p28) were targeted as primer annealing sites for PCR amplification. PCR amplification of the E. ***canis*** p28-7 gene was accomplished with primers 793 (5-GCAGGAGCTGTTGGTTACTC-3') (SEQ ID NO. 16) and 1330 (5'-CCTTCCTCCAAGTTCTATGCC-3') (SEQ ID NO. 17), resulting in a 518-bp PCR product. E. ***canis*** DNA was amplified with primers 793 and 1330 with a thermal cycling profile of 95.degree. C. for 2 min, and. . . followed by a 72.degree. C. extension for 10 min and 4.degree. C. hold. The nucleic acid sequence of the E. ***canis*** PCR product was obtained by sequencing the product directly with primers 793 and 1330.

DETD . . . frame encoding a protein of 170 amino acids, and alignment of the 51-bp sequence obtained from PCR amplification of E. ***canis*** with the DNA sequence of E. ***chaffeensis*** p28 gene revealed a similarity greater than 70%, indicating that the genes were homologous.

DETD . . . PCR product amplified with these primers was sequenced directly with the same primers. The complete DNA sequence for the E.

canis p28-7 gene (SEQ ID NO. 1) is shown in FIG. 1. The p28-7 PCR fragment amplified with these primers contained. . . amino acids from the multiple cloning site and 5' non-coding primer region at the N-terminus. Convalescent-phase antiserum from an E. ***canis*** infected dog recognized the expressed recombinant fusion protein, but did not react with the thioredoxin control (FIG. 2).

DETD Sequence Homology of E. ***Canis*** p28-7 Gene

DETD The nucleic acid sequence of E. ***canis*** p28-7 (834-bp) and the E. ***chaffeensis*** omp-1 family of genes including signal sequences (p28-7, omp-1A, B, C, D, E, and F) were aligned using the Clustal method to examine homology between these genes (alignment not shown). Nucleic acid homology was equally conserved (68.9%) between E. ***canis*** p28-7, E. ***chaffeensis*** p28 and omp-1F. Other putative outer membrane protein genes in the E. ***chaffeensis*** omp-1 family, omp-1D (68.2%), omp-1E (66.7%), omp-1C (64.1%), Cowdria ruminantium map-1 (61.8%), E. ***canis*** 28-kDa protein 1 gene (60%) and 28-kDa protein 2 gene (partial) (59.5%) were also homologous to p28-7. E. ***chaffeensis*** omp-1B had the least nucleic acid homology (45.1%) with E. ***canis*** p28-7.

DETD Alignment of the predicted amino acid sequences of E. ***canis*** P28-7 (SEQ ID NO. 2) and E. ***chaffeensis*** P28 revealed amino acid substitutions resulting in four variable regions (VR). Substitutions or deletions in the amino acid sequence and the locations of variable regions of E. ***canis*** P28-7 and the E. ***chaffeensis*** OMP-1 family were identified (FIG. 3). Amino acid comparison including the signal peptide revealed that E. ***canis*** P28-7 shared the most homology with OMP-1F (68%) of the E. ***chaffeensis*** OMP-1 family, followed by E. ***chaffeensis*** P28 (65.5%), OMP-1E (65.1%), OMP-1D (62.9%), OMP-1C (62.9%), Cowdria ruminantium MAP-1 (59.4%), E. ***canis*** 28-kDa protein 1 (55.6%) and 28-kDa protein 2 (partial) (53.6%), and OMP-1B (43.2%). The phylogenetic relationships based on amino acid sequences show that E. ***canis*** P28-7 and C. ruminantium MAP-1, E. ***chaffeensis*** OMP-1 proteins, and E. ***canis*** 28-kDa proteins 1 and 2 (partial) are related (FIG. 4).

DETD Predicted Surface Probability and Immunoreactivity of E. ***Canis*** P28-7

DETD Analysis of E. ***canis*** P28-7 using hydropathy and hydrophilicity profiles predicted surface-exposed regions on P28-7 (FIG. 6). Eight major surface-exposed regions consisting of 3 to 9 amino acids were identified on E. ***canis*** P28-7 and were similar to the profile of surface-exposed regions on E. ***chaffeensis*** P28 (FIG. 6). Five of the larger surface-exposed regions on E. ***canis*** P28-7 were located in the N-terminal region of the protein. Surface-exposed hydrophilic regions were found in all four of the variable regions of E.

canis P28-7. Ten T-cell motifs were predicted in the P28-7 using the Rothbard-Taylor aligorithm (Rothbard and Taylor, 1988), and high antigenicity of the E. ***canis*** P28-7 was predicted by the Jameson-Wolf antigenicity aligorithm (FIG. 6) (Jameson and Wolf, 1988). Similarities in antigenicity and T-cell motifs were observed between E. ***canis*** P28-7 and E. ***chaffeensis*** P28.

DETD Detection of Homologous Genomic Copies of E. ***Canis*** p28-7 (Gene

DETD Genomic Southern blot analysis of E. ***canis*** DNA completely digested independently with restriction enzymes BanII, EcoRV, HaeII, KpnI, SpeI, which do not have restriction endonuclease sites in. . . nucleotides 34, 43 and 656, revealed the presence of at least three homologous p28-7 gene copies (FIG. 5). Although E. ***canis*** p28-7 has internal AseI internal restriction sites, the DIG-labeled probe used in the hybridization experiment targeted a region of the. . .

DETD PCR Amplification of E. ***Canis*** ECa28SA2 (p28-5), ECa28SA3 (p28-6) Genes and Identification of the Multiple (Gene Locus

DETD Nucleic and Amino Acid Homology of E. ***Canis*** p2g-4p2g-5. p28-6, p28-7 and p28-8 proteins

DETD The nucleic and amino acid sequences of all five E. ***canis*** 28-kDa protein genes were aligned using the Clustal method to examine the homology between these genes. The nucleic acid homology. . . 58 to 75% and a similar amino acid homology of ranging from 67 to 72% was observed between the E. ***canis*** 28-kDa protein gene members (FIG. 9).

DETD . . . protein genes were analyzed for promoter sequences by comparison with consensus Escherichia coli promoter regions and a promoter from E. ***chaffeensis*** (Yu et al., 1997; McClure, 1985). Putative promoter sequences including RBS, -10 and -35 regions were identified in 4 intergenic. . .

DETD N-Terminal Signal Sequence The amino acid sequence analysis revealed that entire E. ***canis*** p28-7 has a deduced molecular mass of 30.5-kDa and the entire p28-6 has a deduced molecular mass of 30.7-kDa. Both. . . N-terminal signal peptide of 23 amino acids (MNCKKILITTALMSLMYYAPSIS, SEQ ID No. 27), which is similar to that predicted for E. ***chaffeensis*** P28 (MNYKKILITSALISLISSLPGV SFS, SEQ ID NO. 28), and the OMP-1 protein family (Yu et al, 1999a; Ohashi et al, 1998b).

DETD . . . cleavage site at amino acid position 25 (MNCKKILITTALISLMYSIPSISSFS, SEQ ID NO. 29) identical to the predicted cleavage site of E. ***chaffeensis*** P28 (SFS) was also present, and would result in a mature p28-7 with a predicted molecular mass of 27.7-kDa. Signal. . .

DETD Proteins of similar molecular mass have been identified and cloned from multiple rickettsial agents including E. ***canis*** , E. ***chaffeensis*** , and C. ruminantium (Reddy et al., 1998; Jongejan et

al., 1993; Ohashi et al., 1998). A single locus in ***Ehrlichia*** ***chaffeensis*** with 6 homologous p28 genes, and 2 loci in E. ***canis*** , each containing some homologous 28-kDa protein genes have been previously described.

DETD The present invention demonstrated the cloning, expression and characterization of genes encoding mature 28-kDa proteins of E. ***canis*** that are homologous to the omp-1 multiple gene family of E. ***chaffeensis*** and the C. ruminantium map-1 gene. Two new 28-kDa protein genes were identidfied, p28-7 and p28-6. Another E. ***canis*** 28-kDa protein gene, p28-5, partially sequenced previously (Reddy et al., 1998), was sequenced completely in the present invention. Also disclosed is the identification and characterization of a single locus in E. ***canis*** containing five E. ***canis*** 28-kDa protein genes (p28-4, p28-5, p28-6, p28-7 and p28-8).

DETD The E. ***canis*** 28-kDa proteins are homologous to E. ***chaffeensis*** OMP-1 family and the MAP-1 protein of C. rumanintium. The most homologous E. ***canis*** 28-kDa proteins (p28-6, p28-7 and p28-8) are sequentially arranged in the locus. Homology of these proteins ranged from 67.5% to 72.3%. Divergence among these 28-kDa proteins was 27.3% to 38.6%. E. ***canis*** 28-kDa proteins p28-4 and p28-5 were the least homologous with homology ranging from 50.9% to 59.4% and divergence of 53.3. . . these regions are surface exposed and subject to selective pressure by the immune system. Conservation of p28-7 among seven E. ***canis*** isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be clonal in North America. Conversely, significant diversity of p28 among E. ***chaffeensis*** isolates has been reported (Yu et al., 1999a).

DETD All of the E. ***canis*** 28-kDa proteins appear to be post translationally processed from a 30-kD protein to a mature 28-kD protein. Recently, a signal sequence was identified on E. ***chaffeensis*** P28 (Yu et al., 1999a), and N-terminal amino acid sequencing has verified that the protein is post-translationally processed resulting in. . . OMP-1F and OMP-1E have also been proposed as leader signal peptides (Ohashi et al., 1998). Signal sequences identified on E. ***chaffeensis*** OMP-1F, OMP-1E and P28 are homologous to the leader sequence of E. ***canis*** 28-kDa protein. Promoter sequences for the p28 genes have not been determined experimentally, but putative promoter regions were identified by comparison with consensus sequences of the RBS, -10 and -35 promoter regions of E. coli and other ***ehrlichia*** (Yu et at., 1997; McClure, 1985). Such promoter sequences would allow each gene to potentially be transcribed and translated, suggesting. . .

DETD The E. ***canis*** 28-kda protein genes were found to exhibit nucleic acid and amino acid sequence homology with the E.

chaffeensis omp-1 gene family and C. ruminantium map-1 gene. Previous studies have identified a 30-kDa protein of E. ***canis*** that reacts with convalescent phase antisera against E. ***chaffeensis*** , but was believed to be antigenically distinct (Rikihisa et al., 1994). Findings based on comparison of amino acid substitutions in four variable regions of E. ***canis*** 28-kDa proteins support this possibility. Together these findings also suggest that the amino acids responsible for the antigenic differences between E. ***canis*** and E. ***chaffeensis*** P28 are located in these variable regions and are readily accessible to the immune system.

DETD It was reported that immunoreactive peptides were located in the variable regions of the 28-kDa proteins of C. ruminantium, E. ***chaffeensis*** and E. ***canis*** (Reddy et al., 1998). Analysis of E. ***canis*** and E. ***chaffeensis*** P28 revealed that all of the variable regions have predicted surface-exposed amino acids. A on study in dogs demonstrated lack of cross protection between E. ***canis*** and E. ***chaffeensis*** (Dawson and Ewing, 1992). This observation may be related to antigenic differences in the variable regions of P28 as well as in other immunologically important antigens of these ***ehrlichia*** species. Another study found that convalescent phase human antisera from E. ***chaffeensis*** -infected patients recognized 29/28-kDa protein(s) of E. ***chaffeensis*** and also reacted with homologous proteins of E. ***canis*** (Chen et al., 1997). Homologous and crossreactive epitopes on the E. ***canis*** 28-kDa protein and E. ***chaffeensis*** P28 appear to be recognized by the immune system.

DETD E. ***canis*** 28-kDa proteins may be important immunoprotective antigens. Several reports have demonstrated that the 30-kDa antigen of E. ***canis*** exhibits strong immunoreactivity (Rikihisa et al., 1994; Rikihisa et al., 1992). Antibodies in convalescent phase antisera from humans and dogs have consistently reacted with proteins in this size range from E. ***chaffeensis*** and E. ***canis*** , suggesting that they may be important immunoprotective antigens (Rikihisa et al., 1994; Chen et al., 1994; Chen et al., 1997). In addition, antibodies to 30, 24 and 21-kDa proteins developed early in the immune response to E. ***canis*** (Rikihisa et al., 1994; Rikihisa et al., 1992), suggesting that these proteins may be especially important in the immune responses. . . Recently, a family of homologous genes encoding outer membrane proteins with molecular masses of 28-kDa have been identified in E. ***chaffeensis*** , and mice immunized with recombinant E. ***chaffeensis*** P28 appeared to have developed immunity against homologous challenge (Ohashi et al., 1998). The P28 of E. ***chaffeensis*** has been demonstrated to be present in the outer membrane, and immunoelectron microscopy has localized the P28 on the surface. . . that it may serve as an adhesin (Ohashi et

al, 1998). It is likely that the 28-kDa proteins of E. ***canis*** identified in this study have the same location and possibly serve a similar function.

DETD Comparison of p28-7 from different strains of E. ***canis*** revealed that the gene is apparently completely conserved. Studies involving E. ***chaffeensis*** have demonstrated immunologic and molecular evidence of diversity. Patients infected with E. ***chaffeensis*** have variable immunoreactivity to the 29/28-kDa proteins, suggesting that there is antigenic diversity (Chen et al., 1997). Recently molecular evidence has been generated to support antigenic diversity in the p28 gene from E. ***chaffeensis*** (Yu et al., 1999a). A comparison of five E. ***chaffeensis*** isolates revealed that two isolates (Sapulpa and St. Vincent) were 100% identical, but three others (Arkansas, Jax, 91HE17) were divergent by as much as 13.4% at the amino acid level. The conservation of E. ***canis*** p28-7 suggests that E. ***canis*** strains found in the United States may be genetically identical, and thus E. ***canis*** 28-kDa protein is an attractive vaccine candidate for canine ***ehrlichiosis*** in the United States. Further analysis of E. ***canis*** isolates outside the United States may provide information regarding the origin and evolution of E. ***canis*** . Conservation of the 28-kDa protein makes it an important potential candidate for reliable serodiagnosis of canine ***ehrlichiosis*** .

DETD The role of multiple homologous genes is not known at this point; however, persistence of E. ***canis*** infections in dogs could conceivably be related to antigenic variation due to variable expression of homologous 28-kDa protein genes, thus enabling E. ***canis*** to evade immune surveillance. Variation of msp-3 genes in A. marginale is partially responsible for variation in the MSP-3 protein, resulting in persistent infections (Alleman et al., 1997). Studies to examine 28-kDa protein gene expression by E. ***canis*** in acutely and chronically infected dogs would provide insight into the role of the 28-kDa protein gene family in persistence. . .

DETD Identification of E. ***Canis*** 28-1, p28-2, p28-3 and p28-9 Genes

DETD The nucleic acid and amino acid sequences of the E. ***canis*** p28 genes were aligned using the Clustal method to examine the homology between these genes. Homology of these proteins ranged from 67.5% to 75%, and divergence among these P28 proteins was 26.9% to 38%. E. ***canis*** P28 proteins P28-1, P28-2, and P28-9 were the least homologous with the other p28 genes ranging from 37% to 49%. . . nucleic acid homology of the nine p28 genes ranged from 28 to 72%. The phylogenetic relationships based on the E. ***canis*** p28 amino acid sequences are shown in FIG. 12.

DETD . . . accession numbers. The GenBank accession numbers for the nucleic acid and amino acid sequences for the complete nine gene E.

canis (Jake strain) p28 gene locus is AF082744. This accession number was originally assigned to p28-7, but has been updated with. . . p28 locus, which includes p28-7. GenBank accession numbers for nucleic acid and amino acid sequences of p28-7 in other E. ***canis*** isolates described in this study are: Louisiana, AF082745; Oklahoma, AF082746; Demon, AF082747; DJ, AF082748; Fuzzy, AF082749; Florida, AF082750.

DETD Multiple bands in the 28-kilodalton range have been observed by immunoblots of convalescent sera from E. ***canis*** infected dogs (Rikihisa et al., 1994), and expression of multiple p28 proteins could be an explanation for this observation. Southern. . .

DETD In this study a single gene locus containing nine tandemly arranged E. ***canis*** p.sup.28 genes encoding homologous, but nonidentical, p28 genes was identified. The nine gene locus included four new p28 genes (FIGS.. . .

DETD The P28s of E. ***canis*** were found to be as closely related to 28-kilodalton proteins of other species such as E. ***chaffeensis*** as they are to themselves (McBride et al., 2000). Differences among the proteins are found primarily in several major hypervariable. . .

DETD Conservation of an E. ***canis*** p28 gene (p28-7) among seven geographically different isolates has been reported (McBride et al., 1999), suggesting that E. ***canis*** may be highly conserved in North America. Similarly, the 120-kDa glycoprotein of E. ***canis*** is also conserved among isolates in the United States (Yu et al., 1997). In contrast, both the 120-kDa and the 28-kDa protein genes of E. ***chaffeensis*** are divergent among isolates (Yu et al., 1999a; Chen et al., 1997). The diversity of the 28-kDa protein gene of E. ***chaffeensis*** appeared to result from point mutations in the hypervariable regions perhaps due to selective immune pressure (Yu et al., 1999a). These data suggest that E. ***canis*** may have been introduced into North America relatively recently, and this may account for the conservation that was observed among isolates. The conservation of p28 genes in E. ***canis*** isolates may provide a n opportunity to develop vaccine and serodiagnostic antigens that are particularly effective for disease prevention and. . .

DETD Troy G. C., et al., (1990) Canine ***ehrlichiosis*** . In Infectious diseases of the dog and cat. Green C. E. (ed). Philadelphia: W.B. Sauders Co.

CLM What is claimed is:

1. An isolated DNA sequence encoding a 30-kilodalton protein of ***Ehrlichia*** ***canis*** , wherein said protein is immunoreactive with anti- ***Ehrlichia*** ***canis*** serum and comprises an amino acid sequence selected from the group consisting of SEQ ID No. 40, 42, 44 and. . .

5. The DNA sequence of claim 1, wherein said DNA is contained in a

single locus of ***Ehrlichia*** ***canis*** .

7. The DNA sequence of claim 6, wherein said locus contains genes encoding homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** .

8. The DNA sequence of claim 7, wherein said homologous 28-kilodalton proteins of ***Ehrlichia*** ***canis*** are selected from the group consisting of p28-1, p28-2, p28-3 and p28-9.

L7 ANSWER 16 OF 30 LIFESCI COPYRIGHT 2003 CSA
AN 2003:29423 LIFESCI
TI Transcriptional Analysis of ***p30*** Major Outer Membrane Protein Genes of ***Ehrlichia*** ***canis*** in Naturally Infected Ticks and Sequence Analysis of ***p30*** - 10 of E. ***canis*** from Diverse Geographic Regions
AU Felek, S.; Greene, R.; Rikihisa, Y.*
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH 43210-1093; E-mail: rikihisa.1@osu.edu
SO Journal of Clinical Microbiology [J. Clin. Microbiol.], (20020200) vol. 41, no. 2, pp. 886-888.
ISSN: 0095-1137.
DT Journal
FS J
LA English
SL English
AB Rhipicephalus sanguineus ticks transmit ***Ehrlichia*** ***canis*** , the etiologic agent of canine ***ehrlichiosis*** . In experimentally infected ticks, only ***p30*** -10 transcript was detected among 22 ***p30*** paralogs encoding immunodominant major outer membrane ***P30*** proteins of E. ***canis*** . The present study revealed transcription of ***p30*** -10 by E. ***canis*** in naturally infected ticks and sequence conservation of ***p30*** -10 genes for E. ***canis*** from diverse geographic regions.
TI Transcriptional Analysis of ***p30*** Major Outer Membrane Protein Genes of ***Ehrlichia*** ***canis*** in Naturally Infected Ticks and Sequence Analysis of ***p30*** - 10 of E. ***canis*** from Diverse Geographic Regions
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transcription of ***p30*** -10 by E. ***canis*** in naturally infected ticks and sequence conservation of ***p30*** -10 genes for E. ***canis*** from diverse geographic regions.

UT Vector-borne diseases; ***Ehrlichiosis*** ; Outer membranes; Membrane proteins; Gene expression; Vectors; ***P30*** protein; ***p30*** gene; ***Ehrlichia*** ***canis*** ; Rhipicephalus sanguineus

L7 ANSWER 17 OF 30 CABA COPYRIGHT 2003 CABI DUPLICATE 5

AN 2002:70420 CABA

DN 20023033646

TI Detection of ***Ehrlichia*** ***canis*** in canine carrier blood and in individual experimentally infected ticks with a ***p30*** -based PCR assay

AU Stich, R. W.; Rikihisa, Y.; Ewing, S. A.; Needham, G. R.; Grover, D. L.; Jittapalapong, S.

CS Department of Veterinary Preventive Medicine, The Ohio State University, 1900 Coffey Rd., Columbus, OH 43210-1092, USA.

SO Journal of Clinical Microbiology, (2002) Vol. 40, No. 2, pp. 540-546. 37 ref.
ISSN: 0095-1137

DT Journal

LA English

AB Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect ***Ehrlichia*** spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique E. ***canis*** gene, ***p30*** , to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for ***Ehrlichia*** ***chaffeensis*** p28 were compared to sequences of primers derived from a sequence conserved among E. ***canis*** isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. ***chaffeensis*** sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The ***p30*** -based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. ***chaffeensis*** , the human granulocytic ***ehrlichiosis*** agent, or ***Ehrlichia*** muris DNA. The assay was used to detect E. ***canis*** in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this ***p30*** -based PCR assay will be useful for experimental

investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

TI Detection of ***Ehrlichia*** ***canis*** in canine carrier blood and in individual experimentally infected ticks with a ***p30*** -based PCR assay.

AB Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect ***Ehrlichia*** spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species. . . The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique E. ***canis*** gene, ***p30*** , to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for ***Ehrlichia*** ***chaffeensis*** p28 were compared to sequences of primers derived from a sequence conserved among E. ***canis*** isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. ***chaffeensis*** sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The ***p30*** -based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. ***chaffeensis*** , the human granulocytic ***ehrlichiosis*** agent, or ***Ehrlichia*** muris DNA. The assay was used to detect E. ***canis*** in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this ***p30*** -based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach. . .

BT ***Canis*** ; Canidae; Fissipeda; carnivores; mammals; vertebrates; Chordata; animals; small mammals; ***Ehrlichia*** ; ***Ehrlichiaceae*** ; Rickettsiales; bacteria; prokaryotes; Rhipicephalus; Ixodidae; Metastigmata; Acari; Arachnida; arthropods; invertebrates

ORGN dogs; ***Ehrlichia*** ***canis*** ; Rhipicephalus sanguineus

L7 ANSWER 18 OF 30 CAPLUS COPYRIGHT 2003 ACS

AN 2001:780727 CAPLUS

DN 135:330480

TI Recombinant intracellular pathogen vaccines: application to mycobacterial disease

IN Horwitz, Marcus A.; Harth, Gunter

PA Regents of the University of California, USA

SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001078774	A2	20011025	WO 2001-US12380	20010416
WO 2001078774	A3	20020328		
US 6471967	B1	20021029	US 2000-550468	20000417
EP 1274453	A2	20030115	EP 2001-927074	20010416

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRAI US 2000-550468 A 20000417
WO 2001-US12380 W 20010416

AB The authors disclose vaccines and immunotherapeutics for preventing intracellular pathogen diseases in mammals. In general, the therapy consists of recombinant attenuated intracellular pathogens that have been transformed to express recombinant extracellular antigens of the same or other intracellular pathogens. In one example, recombinant Mycobacteria bovis was engineered to express the ***p30*** major extracellular protein of M. tuberculosis. Using a guinea pig model of tuberculosis, vaccination with the ***p30*** recombinant bacterium was shown to ameliorate pathol. on aerosol challenge and to reduce bacteremia in lungs and spleen.

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IT Antibacterial agents

Chlamydia
Chlamydia pneumoniae
Coxiella burnetii
Ehrlichia
Ehrlichia ***chaffeensis***
Ehrlichia phagocytophila
Fluoribacter bozemanae
Histoplasma
Legionella longbeachae
Legionella pneumophila
Leishmania
Listeria
Listeria monocytogenes
Mycobacterium BCG
Mycobacterium avium
Mycobacterium bovis
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium tuberculosis
Rickettsia
Rickettsia rickettsi
Rickettsia typhi
Toxoplasma gondii
Trypanosoma cruzi
Vaccines
(attenuated vectors expressing recombinant antigens for vaccination against intracellular pathogens)

L7 ANSWER 19 OF 30 USPATFULL
AN 2001:79141 USPATFULL
TI Immunostimulatory nucleic acid molecules
IN Krieg, Arthur M., Iowa City, IA, United States
Kline, Joel N., Iowa City, IA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
Coley Pharmaceutical Group, Inc., Wellesley, MA, United States (U.S. corporation)
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 6239116 B1 20010529
AI US 1997-960774 19971030 (8)
RLI Continuation-in-part of Ser. No. US 1996-738652, filed on 30 Oct 1996
DT Utility
FS Granted
EXNAM Primary Examiner: Martinell, James

LREP Wolf, Greenfield & Sacks, P.C.
CLMN Number of Claims: 49
ECL Exemplary Claim: 1
DRWN 19 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 3249
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Nucleic acid sequences containing unmethylated CpG dinucleotides that modulate an immune response including stimulating a Th1 pattern of immune activation, cytokine production, NK lytic activity, and B cell proliferation are disclosed. The sequences are also useful a synthetic adjuvant.
SUMM . . . interferon B gene". Proc. Natl. Acad. Sci. USA 89:2150, 1992), TGF-1 (Asiedu, C. K., L. Scott, R. K. Assoian, M. ***Ehrlich*** : "Binding of AP-1/CREB proteins and of MDBP to contiguous sites downstream of the human TGF-B1 gene". Biochim. Biophys. Acta 1219:55,. . .
SUMM . . . that E1A binds to the CREB-binding protein, CBP (Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner: "E1A-associated ***p300*** and CREB-associated CBP belong to a conserved family of coactivators". Cell 77:799, 1994). Human T lymphotropic virus-I (HTLV-1), the retrovirus. . .
DETD . . . spores and drugs (e.g. penicillin). Examples of natural, animal and plant allergens include proteins specific to the following genuses: Canine (***Canis*** familiaris); Dermatophagoides (e.g. Dermatophagoides farinae); Felis (Felis domesticus); Ambrosia (Ambrosia artemiisfolia; Lolium (e.g. Lolium perenne or Lolium multiflorum); Cryptomeria (Cryptomeria. . .

L7 ANSWER 20 OF 30 USPATFULL
AN 2001:44204 USPATFULL
TI Immunostimulatory nucleic acid molecules
IN Krieg, Arthur M., Iowa City, IA, United States
Kline, Joel, Iowa City, IA, United States
Klinman, Dennis, Potomac, MD, United States
Steinberg, Alfred D., Potomac, MD, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
Coley Pharmaceutical Group, Inc., Wellesley, MA, United States (U.S. corporation)
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 6207646 B1 20010327
AI US 1996-738652 19961030 (8)
RLI Continuation of Ser. No. US 1995-386063, filed on 7 Feb 1995
Continuation-in-part of Ser. No. US 1994-276358, filed on 15 Jul 1994,

now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Martinell, James
LREP Wolf, Greenfield & Sacks, P.C.
CLMN Number of Claims: 39
ECL Exemplary Claim: 1
DRWN 19 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 2680
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Nucleic acids containing unmethylated CpG dinucleotides and therapeutic utilities based on their ability to stimulate an immune response and to redirect a Th2 response to a Th1 response in a subject are disclosed.
SUMM . . . interferon B gene". Proc. Natl. Acad. Sci. USA 89:2150, 1992), TGF-.beta.1 (Asiedu, C. K., L. Scott, R. K. Assoian, M. ***Ehrlich*** : "Binding of AP-1/CREB proteins and of MDBP to contiguous sites downstream of the human TGF-B1 gene". Biochim. Biophys. Acta 1219:55,. . .
SUMM . . . that E1A binds to the CREB-binding protein, CBP (Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner: "E1A-associated ***p300*** and CREB-associated CBP belong to a conserved family of coactivators". Cell 77:799, 1994). Human T lymphotropic virus-I (HTLV-1), the retrovirus. . . .
DETD . . . spores and drugs (e.g. penicillin). Examples of natural, animal and plant allergens include proteins specific to the following genera: Canine (***Canis*** familiaris); Dermatophagoides (e.g. Dermatophagoides farinae); Felis (Felis domesticus); Ambrosia (Ambrosia artemiisfolia; Lolium (e.g. Lolium perenne or Lolium multiflorum); Cryptomeria (Cryptomeria. . .

L7 ANSWER 21 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
6
AN 2001:504077 BIOSIS
DN PREV200100504077
TI Transcriptional analysis of ***p30*** major outer membrane multigene family of ***Ehrlichia*** ***canis*** in dogs, ticks, and cell culture at different temperatures.
AU Unver, Ahmet; Ohashi, Norio; Tajima, Tomoko; Stich, Roger W.; Grover, Debra; Rikihisa, Yasuko (1)
CS (1) Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Rd., Columbus, OH, 43210-1093: rikihisa.1@osu.edu USA
SO Infection and Immunity, (October, 2001) Vol. 69, No. 10, pp. 6172-6178. print.

ISSN: 0019-9567.
DT Article
LA English
SL English
AB ***Ehrlichia*** ***canis*** , an obligatory intracellular bacterium of monocytes and macrophages, causes canine monocytic ***ehrlichiosis*** . E. ***canis*** immunodominant 30-kDa major outer membrane proteins are encoded by a polymorphic multigene family consisting of more than 20 paralogs. In the present study, we analyzed the mRNA expression of 14 paralogs in experimentally infected dogs and Rhipicephalus sanguineus ticks by reverse transcription-PCR using gene-specific primers followed by Southern blotting. Eleven out of 14 paralogs in E. ***canis*** were transcribed in increasing numbers and transcription levels, while the mRNA expression of the 3 remaining paralogs was not detected in blood monocytes of infected dogs during the 56-day postinoculation period. Three different groups of R. sanguineus ticks (adult males and females and nymphs) were separately infected with E. ***canis*** by feeding on the infected dogs. In these pools of acquisition-fed ticks as well as in the transmission-fed adult ticks, the transcript from only one paralog was detected, suggesting the predominant transcription of that paralog or the suppression of the remaining paralogs in ticks. Expression of the same paralog was higher whereas expression of the remaining paralogs was lower in E. ***canis*** cultivated in dog monocyte cell line DH82 at 25degreeC than in E. ***canis*** cultivated at 37degreeC. Analysis of differential expression of ***p30*** multigenes in dogs, ticks, or monocyte cell cultures would help in understanding the role of these gene products in pathogenesis and E. ***canis*** transmission as well as in designing a rational vaccine candidate immunogenic against canine ***ehrlichiosis*** .
TI Transcriptional analysis of ***p30*** major outer membrane multigene family of ***Ehrlichia*** ***canis*** in dogs, ticks, and cell culture at different temperatures.
AB ***Ehrlichia*** ***canis*** , an obligatory intracellular bacterium of monocytes and macrophages, causes canine monocytic ***ehrlichiosis*** . E. ***canis*** immunodominant 30-kDa major outer membrane proteins are encoded by a polymorphic multigene family consisting of more than 20 paralogs. In. . . Rhipicephalus sanguineus ticks by reverse transcription-PCR using gene-specific primers followed by Southern blotting. Eleven out of 14 paralogs in E. ***canis*** were transcribed in increasing numbers and transcription levels, while the mRNA expression of the 3 remaining paralogs was not detected. . . postinoculation period. Three different groups of R. sanguineus ticks (adult males and females and nymphs) were separately infected with E. ***canis*** by feeding on the infected dogs. In these pools of acquisition-fed ticks as well as in the transmission-fed adult ticks,. . . paralogs in ticks.

Expression of the same paralog was higher whereas expression of the remaining paralogs was lower in E. ***canis*** cultivated in dog monocyte cell line DH82 at 25degreeC than in E. ***canis*** cultivated at 37degreeC. Analysis of differential expression of ***p30*** multigenes in dogs, ticks, or monocyte cell cultures would help in understanding the role of these gene products in pathogenesis and E. ***canis*** transmission as well as in designing a rational vaccine candidate immunogenic against canine ***ehrlichiosis*** .

IT . . .

blood: blood and lymphatics; macrophage: blood and lymphatics, immune system; monocyte: blood and lymphatics, immune system

IT Diseases

canine monocytic ***ehrlichiosis*** : bacterial disease

IT Chemicals & Biochemicals

canine ***ehrlichiosis*** vaccine: immunostimulant - drug; gene-specific primers; major outer membrane proteins: 30 kilodaltons; ***p30*** major outer membrane multigene family

ORGN . . .

Carnivora, Mammalia, Vertebrata, Chordata, Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name

DH82 cell line (Canidae); ***Ehrlichia*** ***canis*** (Rickettsiaceae): pathogen; Rhipicephalus sanguineus [tick] (Acarina): acquisition-fed, adult, female, male, nymph, transmission-fed; dog (Canidae): host; tick (Acarina): host

ORGN Organism. . .

L7 ANSWER 22 OF 30 CAPLUS COPYRIGHT 2003 ACS

AN 2001:473743 CAPLUS

DN 138:118175

TI Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** . [Erratum to document cited in CA135:340042]

AU Ohashi, Norio; Rikihisa, Yasuko; Unver, Ahmet

CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA

SO Infection and Immunity (2001), 69(7), 4702
CODEN: INFIBR; ISSN: 0019-9567

PB American Society for Microbiology

DT Journal

LA English

AB On page 2083, abstr., line 2, "in humans and dogs, resp." should read "in dogs and humans, resp.".

TI Analysis of transcriptionally active gene clusters of major outer membrane

protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** . [Erratum to document cited in CA135:340042]

ST erratum ***Ehrlichia*** major outer membrane protein gene cluster sequence; ***Ehrlichia*** major outer membrane protein gene cluster sequence erratum; transcription major outer membrane protein gene cluster ***Ehrlichia*** erratum

IT Genetic element
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(IGS (intergenic spacer); anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Porins
Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(MOMP (major outer membrane protein), paralogs; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT DNA sequences
Dog (***Canis*** familiaris)
Ehrlichia ***canis***
Ehrlichia ***chaffeensis***
Gene dosage
Human
Protein sequences
Transcription, genetic
(anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1A; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1B; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1C; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1D; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1E; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1F; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1H; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1M; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1N; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1P; anal. of transcriptionally active gene clusters of major outer

membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1Q; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1S; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1T; anal. of transcriptionally active gene clusters of major outer. membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1U; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1V; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1W; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1X; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1Y; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(omp-1Z; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(p28-1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(p28-2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(p28; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -10; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -11; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -12; anal. of transcriptionally active gene clusters of

major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -13; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -14; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -15; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -16; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -17; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -18; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -19; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -20; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -3; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -4; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -5; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -6; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -7; anal. of transcriptionally active gene clusters of

major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -8; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** -9; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30*** ; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***p30a*** ; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Transport proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(preprotein-transporting; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(secA; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(u1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(u2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(u3; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(u4; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(u5; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(un1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(un2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(un3; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(un4; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(un5; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT 202833-62-3 202833-63-4 202833-64-5 202833-65-6 202833-66-7
211371-48-1 215311-72-1 215311-73-2 221625-03-2 221625-07-6
221625-08-7 221625-09-8 221625-10-1 221625-11-2 221625-14-5
221625-17-8 221625-20-3 229963-41-1 273716-44-2 296809-60-4
296809-63-7 296809-67-1 296809-68-2 296809-70-6 296809-71-7
316196-93-7 316196-94-8 370153-97-2 370153-98-3 370153-99-4
370154-00-0 370154-01-1 370154-02-2 370154-03-3 370154-04-4
370154-05-5 370154-06-6 370154-07-7 370154-08-8 370154-09-9
370154-10-2 370154-11-3 370154-12-4 370154-13-5 370154-14-6
370154-15-7 370154-16-8 370154-17-9 370154-18-0 370154-19-1
370154-20-4 370154-21-5 370154-22-6 370154-23-7 370154-24-8
370154-25-9 370154-26-0 370154-27-1
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT 9032-04-6, Phosphoribosylaminoimidazole carboxylase
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

IT 202913-78-8, DNA (***Ehrlichia*** ***chaffeensis*** strain Arkansas major outer membrane protein OMP-1 multigene cluster)
216654-49-8, DNA (***Ehrlichia*** ***canis*** strain Oklahoma major outer membrane protein ***P30*** multigene cluster 1)
331227-94-2, GenBank AF324792
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** (Erratum))

L7 ANSWER 23 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

7
AN 2002:7769 BIOSIS
DN PREV200200007769
TI Identification of a p28 Gene in ***Ehrlichia*** ewingii: Evaluation of gene for use as a target for a species-specific PCR diagnostic assay.
AU Gusa, Asiya A.; Buller, Richard S.; Storch, Gregory A.; Huycke, Mark M.; Machado, Linda J.; Slater, Leonard N.; Stockham, Steven L.; Massung, Robert F. (1)
CS (1) Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA, 30333: rfm2@cdc.gov USA
SO Journal of Clinical Microbiology, (November, 2001) Vol. 39, No. 11, pp. 3871-3876. print.
ISSN: 0095-1137.
DT Article
LA English
AB PCR was used to amplify a 537-bp region of an ***Ehrlichia*** ewingii gene encoding a homologue of the 28-kDa major antigenic protein (P28) of ***Ehrlichia*** ***chaffeensis*** . The E. ewingii p28 gene homologue was amplified from DNA extracted from whole blood obtained from four humans and one canine with confirmed cases of infection. Sequencing of the PCR products (505 bp) revealed a partial gene with homology to outer membrane protein genes from ***Ehrlichia*** and Cowdria spp.: ***p30*** of ***Ehrlichia*** ***canis*** (ltoreq71.3%), p28 of E. ***chaffeensis*** (ltoreq68.3%), and map1 of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with ltoreq69.1% identity to P28 of E. ***chaffeensis*** , ltoreq67.3% identity to ***P30*** of E. ***canis*** , and ltoreq63.1% identity to MAP1 of C. ruminantium. Primers were selected from the E. ewingii p28 sequence and used to develop a species-specific PCR diagnostic assay. The p28 PCR assay amplified the expected 215-bp product from DNA that was extracted from EDTA-treated blood from each of the confirmed E. ewingii infections that were available. The assay did not produce PCR products with DNA extracted from E. ***chaffeensis*** -, E. ***canis*** -, or E. phagocytophila-infected samples, confirming the specificity of the p28 assay for E. ewingii. The sensitivity of the E. ewingii-specific PCR assay was evaluated and determined to detect as few as 38 copies of the p28 gene.
TI Identification of a p28 Gene in ***Ehrlichia*** ewingii: Evaluation of gene for use as a target for a species-specific PCR diagnostic assay.
AB PCR was used to amplify a 537-bp region of an ***Ehrlichia*** ewingii gene encoding a homologue of the 28-kDa major antigenic protein (P28) of ***Ehrlichia*** ***chaffeensis*** . The E. ewingii p28 gene homologue was amplified from DNA extracted from whole blood obtained from four humans and one. . . infection. Sequencing of the PCR products (505 bp)

revealed a partial gene with homology to outer membrane protein genes from ***Ehrlichia*** and Cowdria spp.: ***p30*** of ***Ehrlichia*** ***canis*** (ltoreq71.3%), p28 of E. ***chaffeensis*** (ltoreq68.3%), and map1 of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with ltoreq69.1% identity to P28 of E. ***chaffeensis*** , ltoreq67.3% identity to ***P30*** of E. ***canis*** , and ltoreq63.1% identity to MAP1 of C. ruminantium. Primers were selected from the E. ewingii p28 sequence and used to. . . the confirmed E. ewingii infections that were available. The assay did not produce PCR products with DNA extracted from E. ***chaffeensis*** -, E. ***canis*** -, or E. phagocytophila-infected samples, confirming the specificity of the p28 assay for E. ewingii. The sensitivity of the E. ewingii-specific. . .

ORGN . . .
Primates, Mammalia, Vertebrata, Chordata, Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Cowdria spp. (Rickettsiaceae): pathogen; ***Ehrlichia*** ***canis*** (Rickettsiaceae): pathogen; ***Ehrlichia*** ***chaffeensis*** (Rickettsiaceae): pathogen; ***Ehrlichia*** ewingii (Rickettsiaceae): pathogen; canine (Canidae): host; human (Hominidae): host, patient

ORGN Organism Superterms
Animals; Bacteria; Carnivores; Chordates; Eubacteria; Humans; Mammals;. . .

GEN ***Ehrlichia*** ***canis*** ***p30*** gene (Rickettsiaceae); ***Ehrlichia*** ewingii p28 gene (Rickettsiaceae)

L7 ANSWER 24 OF 30 CAPLUS COPYRIGHT 2003 ACS
AN 2001:240579 CAPLUS
DN 135:340042
TI Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***
AU Ohashi, Norio; Rikihisa, Yasuko; Unver, Ahmet
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SO Infection and Immunity (2001), 69(4), 2083-2091
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** are

tick-borne obligatory intramonocytic ***ehrlichiae*** that cause febrile systemic illness in dogs and humans, resp. The current study analyzed the pleomorphic multigene family encoding approx. 30-kDa major outer membrane proteins (OMPs) of E. ***canis*** and E. ***chaffeensis*** . Upstream from secA and downstream of hypothetical transcriptional regulator, 22 paralogs of the omp gene family were found to be tandemly arranged except for one or two genes with opposite orientations in a 28- and a 27-kb locus in the E. ***canis*** and E. ***chaffeensis*** genomes, resp. Each locus consisted of three highly repetitive regions with four nonrepetitive intervening regions. E. ***canis*** , in addn., had a 6.9-kb locus which contained a repeat of three tandem paralogs in the 28-kb locus. These total 47 paralogous and orthologous genes encoded OMPs of approx. 30 to 35 kDa consisting of several hypervariable regions alternating with conserved regions. In the 5' -end half of the 27-kb locus or the 28-kb locus of each ***Ehrlichia*** species, 14 paralogs were linked by short intergenic spaces ranging from -8 bp (overlapped) to 27 bp, and 8 remaining paralogs in the 3' -end half were connected by longer intergenic spaces ranging from 213 to 632 bp. All 22 paralogs, five unknown genes, and secA in the omp cluster in E. ***canis*** were transcriptionally active in the monocyte culture, and the paralogs with short intergenic spaces were cotranscribed with their adjacent genes, including the resp. intergenic spaces at both the 5' and the 3' sides. Although omp genes are diverse, our results suggest that the gene organization of the clusters and the gene locus are conserved between two species of ***Ehrlichia*** to maintain a unique transcriptional mechanism for adaptation to environmental changes common to them.

RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***

AB ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** are tick-borne obligatory intramonocytic ***ehrlichiae*** that cause febrile systemic illness in dogs and humans, resp. The current study analyzed the pleomorphic multigene family encoding approx. 30-kDa major outer membrane proteins (OMPs) of E. ***canis*** and E. ***chaffeensis*** . Upstream from secA and downstream of hypothetical transcriptional regulator, 22 paralogs of the omp gene family were found to be tandemly arranged except for one or two genes with opposite orientations in a 28- and a 27-kb locus in the E. ***canis*** and E. ***chaffeensis*** genomes, resp. Each locus consisted of three highly repetitive regions with four nonrepetitive intervening regions. E. ***canis*** , in addn., had a 6.9-kb locus which contained a repeat of three tandem paralogs in the 28-kb locus. These total 47 paralogous and

orthologous genes encoded OMPs of approx. 30 to 35 kDa consisting of several hypervariable regions alternating with conserved regions. In the 5' -end half of the 27-kb locus or the 28-kb locus of each ***Ehrlichia*** species, 14 paralogs were linked by short intergenic spaces ranging from -8 bp (overlapped) to 27 bp, and 8 remaining paralogs in the 3' -end half were connected by longer intergenic spaces ranging from 213 to 632 bp. All 22 paralogs, five unknown genes, and secA in the omp cluster in E. ***canis*** were transcriptionally active in the monocyte culture, and the paralogs with short intergenic spaces were cotranscribed with their adjacent genes, including the resp. intergenic spaces at both the 5' and the 3' sides. Although omp genes are diverse, our results suggest that the gene organization of the clusters and the gene locus are conserved between two species of ***Ehrlichia*** to maintain a unique transcriptional mechanism for adaptation to environmental changes common to them.

ST ***Ehrlichia*** major outer membrane protein gene cluster sequence; transcription major outer membrane protein gene cluster ***Ehrlichia***

IT Genetic element
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(IGS (intergenic spacer); anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Glycoproteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(MOMP (major outer membrane protein), paralogs; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Porins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(MOMP (major outer membrane protein), paralogs; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT DNA sequences
Ehrlichia ***canis***
Ehrlichia ***chaffeensis***
Gene dosage
Protein sequences
Transcription, genetic
(anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis***

and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1A; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1B; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1C; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1D; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1E; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1F; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1H; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); PROC (Process)
(omp-1M; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1N; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1P; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1Q; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1S; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1T; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1U; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)
IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(omp-1V; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis***

and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(omp-1W; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(omp-1X; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(omp-1Y; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(omp-1Z; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(p28-1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(p28-2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(p28; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); PROC (Process)
(***p30*** -10; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -11; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -12; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -13; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -14; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -15; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -16; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -17; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia***

canis and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -18; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -19; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -20; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -3; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(***p30*** -4; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); PROC (Process)
(***p30*** -5; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -6; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -7; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -8; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** -9; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30*** ; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(***p30a*** ; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Transport proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(preprotein-transporting; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in

Ehrlichia ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(secA; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(u1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(u2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(u3; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(u4; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(u5; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(un1; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); PROC (Process)
(un2; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(un3; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(un4; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(un5; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT 202833-62-3 202833-63-4 202833-64-5 202833-65-6 202833-66-7
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370154-05-5 370154-06-6 370154-07-7 370154-08-8 370154-09-9
370154-10-2 370154-11-3 370154-12-4 370154-13-5 370154-14-6
370154-15-7 370154-16-8 370154-17-9 370154-18-0 370154-19-1
370154-20-4 370154-21-5 370154-22-6 370154-23-7 370154-24-8
370154-25-9 370154-26-0 370154-27-1
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT 9032-04-6, Phosphoribosylaminoimidazole carboxylase
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

IT 202913-78-8, DNA (***Ehrlichia*** ***chaffeensis*** strain Arkansas major outer membrane protein OMP-1 multigene cluster)
216654-49-8, DNA (***Ehrlichia*** ***canis*** strain Oklahoma major outer membrane protein ***P30*** multigene cluster 1)
331227-94-2, GenBank AF324792
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(nucleotide sequence; anal. of transcriptionally active gene clusters of major outer membrane protein multigene family in ***Ehrlichia*** ***canis*** and E. ***chaffeensis***)

L7 ANSWER 25 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:379120 BIOSIS
DN PREV200000379120
TI Characterization and expression analysis of ***p30*** multigenes encoding major outer membrane proteins identified within two regions of ***Ehrlichia*** ***canis*** genome.
AU Ohashi, N. (1); Unver, A. (1); Rikihisa, Y. (1)
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 242-243. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology Los Angeles, California, USA May 21-25, 2000 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English
SL English
TI Characterization and expression analysis of ***p30*** multigenes encoding major outer membrane proteins identified within two regions of ***Ehrlichia*** ***canis*** genome.
IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics); Evolution and Adaptation; Infection
IT Diseases
canine ***ehrlichiosis*** : bacterial disease
IT Chemicals & Biochemicals
DNA: analysis; outer membrane proteins; proteins; ***Ehrlichia*** ***canis*** ***p30*** gene (Rickettsiaceae): expression, multiple
ORGN Super Taxa
Canidae: Carnivora, Mammalia, Vertebrata, Chordata, Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Ehrlichia ***canis*** (Rickettsiaceae): pathogen; canine

(Canidae)
ORGN Organism Superterms
Animals; Bacteria; Carnivores; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Vertebrates

L7 ANSWER 26 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:379119 BIOSIS
DN PREV200000379119
TI Expression analysis of multiple ***p30*** genes of ***Ehrlichia*** ***canis*** in dogs and ticks.
AU Unver, A. (1); Tajima, T. (1); Ohashi, N. (1); Rikihisa, Y. (1); Stich, R. W. (1)
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 242. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology Los Angeles, California, USA May 21-25, 2000 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English
SL English
TI Expression analysis of multiple ***p30*** genes of ***Ehrlichia*** ***canis*** in dogs and ticks.
IT . . .
Biophysics); Infection; Veterinary Medicine (Medical Sciences); Vector Biology
IT Chemicals & Biochemicals
mRNA [messenger RNA]: expression; outer membrane proteins; proteins; ***Ehrlichia*** ***canis*** ***p30*** gene (Rickettsiaceae): expression, multiple
ORGN . . .
Arthropoda, Invertebrata, Animalia; Canidae: Carnivora, Mammalia, Vertebrata, Chordata, Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Ehrlichia ***canis*** (Rickettsiaceae): pathogen; dogs (Canidae): host; ticks (Acarina): vector
ORGN Organism Superterms
Animals; Arthropods; Bacteria; Carnivores; Chelicerates; Chordates; Eubacteria; Invertebrates; Mammals;. . .

L7 ANSWER 27 OF 30 CAPLUS COPYRIGHT 2003 ACS
AN 1999:219938 CAPLUS
DN 130:249405

TI Outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***

IN Rikihisa, Yasuko; Ohashi, Noris
PA The Ohio State University, USA

SO PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9913720	A1	19990325	WO 1998-US19600	19980918
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2304256	AA	19990325	CA 1998-2304256	19980918
AU 9895719	A1	19990405	AU 1998-95719	19980918
AU 748357	B2	20020606		
EP 1026949	A1	20000816	EP 1998-949384	19980918
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRAI US 1997-59353P P 19970919
WO 1998-US19600 W 19980918

AB Outer membrane proteins of ***Ehrlichia*** that can be used in the serodiagnosis of ***ehrlichiosis*** in man and in Canidae and the genes encoding them are characterized. The genes for a family of OMP-1 proteins of E. ***chaffeensis*** and a ***P30*** family of proteins of E. ***canis*** are cloned. Sequences of genes and proteins of E. ***chaffeensis*** OMP-1, OMP-1A, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, and OMP-1Z are reported. Similarly, sequence of genes and proteins of E. ***canis*** ***P30*** , ***P30*** -a, ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, and ***P30*** -10, referred to hereinafter as the ***P30*** family. The present invention also relates to an assay for diagnosing ***ehrlichiosis*** in humans using a recombinant outer membrane protein of E. ***chaffeensis*** , particularly OMP-1. The present invention also relates to an assay for diagnosing ***ehrlichiosis*** in humans and members of the family Canidae using a recombinant outer membrane protein of E. ***canis*** , particularly ***P30*** .

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Outer membrane proteins of ***Ehrlichia*** ***canis*** and

Ehrlichia ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***

AB Outer membrane proteins of ***Ehrlichia*** that can be used in the serodiagnosis of ***ehrlichiosis*** in man and in Canidae and the genes encoding them are characterized. The genes for a family of OMP-1 proteins of E. ***chaffeensis*** and a ***P30*** family of proteins of E. ***canis*** are cloned. Sequences of genes and proteins of E. ***chaffeensis*** OMP-1, OMP-1A, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, and OMP-1Z are reported. Similarly, sequence of genes and proteins of E. ***canis*** ***P30*** , ***P30*** -a, ***P30*** -1, ***P30*** -2, ***P30*** -3, ***P30*** -4, ***P30*** -5, ***P30*** -6, ***P30*** -7, ***P30*** -8, ***P30*** -9, and ***P30*** -10, referred to hereinafter as the ***P30*** family. The present invention also relates to an assay for diagnosing ***ehrlichiosis*** in humans using a recombinant outer membrane protein of E. ***chaffeensis*** , particularly OMP-1. The present invention also relates to an assay for diagnosing ***ehrlichiosis*** in humans and members of the family Canidae using a recombinant outer membrane protein of E. ***canis*** , particularly ***P30*** .

ST ***ehrlichiosis*** serodiagnosis outer membrane protein antigens; ***P30*** isoforms ***Ehrlichia*** gene cloning; OMP1 isoforms ***Ehrlichia*** gene cloning

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(OMP1 (outer membrane protein 1), isoforms; outer membrane proteins of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and genes encoding them and diagnosis of ***Ehrlichiosis***)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***P30*** , isoforms; outer membrane proteins of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and genes encoding them and diagnosis of ***Ehrlichiosis***)

IT Vaccines
(***ehrlichiosis*** , outer membrane protein antigens for; outer membrane proteins of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and genes encoding them and diagnosis of ***Ehrlichiosis***)

IT Immunoassay
(for diagnosis of ***ehrlichiosis*** , outer membranes as antigens for; outer membrane proteins of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and genes encoding them and diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1B, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1C, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1D, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1E, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1F, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1R, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1S, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1T, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1U, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1V, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1W, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1X, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(omp-1Y, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and

the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp-1Z, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(omp1, for OMP1 isoform of ***Ehrlichia*** ***chaffeensis*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT DNA sequences
Ehrlichia ***chaffeensis***
Protein sequences
(outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT Antibodies
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(to outer membrane proteins of ***Ehrlichia*** ; outer membrane proteins of ***Ehrlichia*** ***canis*** and E. ***chaffeensis*** and genes encoding them and diagnosis of ***Ehrlichiosis***)

IT 202833-61-2 202833-62-3 202833-63-4 202833-64-5 202833-65-6 202833-66-7 202833-67-8
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT 200662-13-1 215311-72-1 215311-75-4 221625-01-0 221625-02-1 221625-03-2 221625-05-4 221625-06-5 221625-07-6 221625-08-7 221625-09-8 221625-10-1 221625-11-2 221625-12-3 221625-13-4 221625-14-5 221625-15-6 221625-16-7 221625-17-8 221625-18-9 221625-19-0 221625-20-3 221625-21-4 221625-22-5
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(amino acid sequence; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

IT 202913-78-8, GenBank U72291 202913-79-9, GenBank AF021338
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)
IT 216654-47-6 216654-48-7 216654-49-8 221541-95-3 221541-98-6 221542-00-3 221542-01-4 221542-02-5 221542-03-6 221542-04-7 221542-05-8 221542-06-9 221542-07-0 221542-08-1 221542-09-2 221542-10-5 221542-11-6 221542-12-7 221542-70-7 221542-71-8 221542-72-9 221542-73-0 221542-74-1 221542-75-2 221542-76-3 221542-77-4 221542-78-5 221542-79-6 221542-80-9
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nucleotide sequence; outer membrane proteins of ***Ehrlichia*** ***canis*** and ***Ehrlichia*** ***chaffeensis*** and the genes encoding them and the diagnosis of ***Ehrlichiosis***)

L7 ANSWER 28 OF 30 CAPLUS COPYRIGHT 2003 ACS
AN 1999:809483 CAPLUS
DN 132:277873
TI Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-positive and -negative human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens
AU Unver, Ahmet; Rikihisa, Yasuko; Ohashi, Norio; Cullman, Louis C.; Buller, Richard; Storch, Gregory A.
CS Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SO Journal of Clinical Microbiology (1999), 37(12), 3888-3895
CODEN: JCMIDW; ISSN: 0095-1137
PB American Society for Microbiology
DT Journal
LA English
AB Human monocytic ***ehrlichiosis*** is an emerging infectious disease caused by ***Ehrlichia*** ***chaffeensis*** , a gram-neg. obligatory intracellular bacterium closely related to E. ***canis*** . The immunoreactive recombinant fusion proteins rP28 and rP30 have become available after cloning and expressing of the 28- and 30-kDa major outer membrane protein genes of E. ***chaffeensis*** and E. ***canis*** , resp. Western immunoblotting was performed to analyze the antibody responses of the 37 E. ***chaffeensis*** indirect fluorescent-antibody assay (IFA)-pos. and 20 IFA-neg. serum specimens with purified whole organisms, rP28, and rP30. All IFA-neg. sera were neg. with purified whole organisms, rP28, or rP30 by Western immunoblot anal. (100% relative

diagnostic specificity). Of 37 IFA-pos. sera, 34 sera reacted with any native proteins of E. ***chaffeensis*** ranging from 44 to 110 kDa, and 30 sera reacted with 44- to 110-kDa native E. ***canis*** antigens. The 28-kDa E. ***chaffeensis*** and 30-kDa E. ***canis*** native proteins were recognized by 25 IFA-pos. sera. Fifteen IFA-pos. sera reacted with rP28 by Western blot anal., whereas 34 IFA-pos. sera reacted with rP30 (92% relative diagnostic specificity), indicating that rP30 is more sensitive than rP28 for detecting the antibodies in IFA-pos. sera. These 34 IFA-pos. sera were pos. by the dot blot assay with rP30, distinguishing them from IFA-neg. sera. Except for three rP30-neg. but IFA-pos. specimens that instead showed an E. ewingii infection-like profile by Western immunoblotting, the results of Western and dot blot assays with rP30 matched 100% with the IFA test results. Densitometric anal. of dot blot reactions showed a pos. correlation between the dot d. and the IFA titer. These results suggest that rP30 antigen would provide a simple, consistent, and rapid serodiagnosis for human monocytic ***ehrlichiosis*** .

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-positive and -negative human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens

AB Human monocytic ***ehrlichiosis*** is an emerging infectious disease caused by ***Ehrlichia*** ***chaffeensis*** , a gram-neg. obligatory intracellular bacterium closely related to E. ***canis*** . The immunoreactive recombinant fusion proteins rP28 and rP30 have become available after cloning and expressing of the 28- and 30-kDa major outer membrane protein genes of E. ***chaffeensis*** and E. ***canis*** , resp. Western immunoblotting was performed to analyze the antibody responses of the 37 E. ***chaffeensis*** indirect fluorescent-antibody assay (IFA)-pos. and 20 IFA-neg. serum specimens with purified whole organisms, rP28, and rP30. All IFA-neg. sera were neg. with purified whole organisms, rP28, or rP30 by Western immunoblot anal. (100% relative diagnostic specificity). Of 37 IFA-pos. sera, 34 sera reacted with any native proteins of E. ***chaffeensis*** ranging from 44 to 110 kDa, and 30 sera reacted with 44- to 110-kDa native E. ***canis*** antigens. The 28-kDa E. ***chaffeensis*** and 30-kDa E. ***canis*** native proteins were recognized by 25 IFA-pos. sera. Fifteen IFA-pos. sera reacted with rP28 by Western blot anal., whereas 34 IFA-pos. sera reacted with rP30 (92% relative diagnostic specificity), indicating that rP30 is more sensitive than rP28 for detecting the antibodies in IFA-pos. sera. These 34 IFA-pos. sera were pos. by the dot blot assay with rP30, distinguishing them from IFA-neg. sera. Except for three rP30-neg. but IFA-pos. specimens that instead showed an E. ewingii infection-like

profile by Western immunoblotting, the results of Western and dot blot assays with rP30 matched 100% with the IFA test results. Densitometric anal. of dot blot reactions showed a pos. correlation between the dot d. and the IFA titer. These results suggest that rP30 antigen would provide a simple, consistent, and rapid serodiagnosis for human monocytic ***ehrlichiosis*** .

ST ***Ehrlichia*** P28 ***P30*** protein antibody

IT Proteins, specific or class
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***P30*** ; Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-pos. and -neg. human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens)

IT ***Ehrlichia*** ***canis***
Ehrlichia ***chaffeensis***
(Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-pos. and -neg. human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens)

IT Antibodies
RL: ANT (Analyte); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); FORM (Formation, nonpreparative); USES (Uses)
(Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-pos. and -neg. human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens)

IT Blood analysis
Diagnosis
(Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-pos. and -neg. human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens in relation to)

IT Proteins, specific or class
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(p28; Western and dot blotting analyses of ***Ehrlichia*** ***chaffeensis*** indirect fluorescent-antibody assay-pos. and -neg. human sera by using native and recombinant E. ***chaffeensis*** and E. ***canis*** antigens)

L7 ANSWER 29 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:324751 BIOSIS
DN PREV199900324751
TI Characterization of ***p30*** multigene family of ***Ehrlichia*** ***canis*** .

AU Ohashi, N. (1); Rikihisa, Y. (1)
CS (1) Ohio State University, Columbus, OH USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 233.
Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English
TI Characterization of ***p30*** multigene family of ***Ehrlichia*** ***canis*** .
IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics)
IT Diseases
ehrlichiosis : bacterial disease
IT Chemicals & Biochemicals
open reading frame; ***Ehrlichia*** - ***canis*** ***p30*** gene (Rickettsiaceae)
IT Alternate Indexing
Ehrlichiosis (MeSH)
ORGN . . .
Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
dog (Canidae); ***Ehrlichia*** - ***canis*** (Rickettsiaceae): pathogen; Escherichia-coli (Enterobacteriaceae): expression system
ORGN Organism Superterms
Animals; Bacteria; Carnivores; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates;. . .

L7 ANSWER 30 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
8
AN 1998:435123 BIOSIS
DN PREV199800435123
TI Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of ***Ehrlichia*** ***canis*** and application of the recombinant protein for serodiagnosis.
AU Ohashi, Norio; Unver, Ahmet; Zhi, Ning; Rikihisa, Yasuko (1)
CS (1) Dep. Veterinary Biosciences, Coll. Veterinary Med., Ohio State Univ., 1925 Coffey Rd., Columbus, OH 43210-1093 USA
SO Journal of Clinical Microbiology, (Sept., 1998) Vol. 36, No. 9, pp.

2671-2680.
ISSN: 0095-1137.
DT Article
LA English
AB A 30-kDa major outer membrane protein of ***Ehrlichia*** ***canis*** , the agent of canine ***ehrlichiosis*** , is the major antigen recognized by both naturally and experimentally infected dog sera. The protein cross-reacts with a serum against a recombinant 28-kDa protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of ***Ehrlichia*** ***chaffeensis*** . Two DNA fragments of E. ***canis*** were amplified by PCR with two primer pairs based on the sequences of E. ***chaffeensis*** omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of E. ***canis*** . Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E. ***canis*** genome. Three copies of the entire gene (***p30*** , ***p30*** -1, and ***p30a***) were cloned and sequenced from the E. ***canis*** genomic DNA. The open reading frames of the two copies (***p30*** and ***p30*** -1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E. ***canis*** 30-kDa protein genes and the E. ***chaffeensis*** omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp., p44 from the agent of human granulocytic ***ehrlichiosis*** , msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. ***canis*** 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E. ***canis*** 30-kDa proteins are closely related but that the third 30-kDa protein is not. The ***p30*** gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified E. ***canis*** . Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 of E. ***canis*** . To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ***ehrlichiosis*** , the immunoreactions between rP30 and the whole purified E. ***canis*** antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IFA-positive and -negative plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ***ehrlichiosis*** . Cloning of multigenes encoding the 30-kDa major

outer membrane proteins of E. ***canis*** will greatly facilitate understanding pathogenesis and immunologic study of canine ***ehrlichosis*** and provide a useful tool for phylogenetic analysis.

TI Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of ***Ehrlichia*** ***canis*** and application of the recombinant protein for serodiagnosis.

AB A 30-kDa major outer membrane protein of ***Ehrlichia*** ***canis*** , the agent of canine ***ehrlichiosis*** , is the major antigen recognized by both naturally and experimentally infected dog sera. The protein cross-reacts with a serum against a recombinant 28-kDa protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of ***Ehrlichia*** ***chaffeensis*** . Two DNA fragments of E. ***canis*** were amplified by PCR with two primer pairs based on the sequences of E. ***chaffeensis*** omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of E. ***canis*** . Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E. ***canis*** genome. Three copies of the entire gene (***p30*** , ***p30*** -1, and ***p30a***) were cloned and sequenced from the E. ***canis*** genomic DNA. The open reading frames of the two copies (***p30*** and ***p30*** -1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E. ***canis*** 30-kDa protein genes and the E. ***chaffeensis*** omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp., p44 from the agent of human granulocytic ***ehrlichiosis*** , msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. ***canis*** 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E. ***canis*** 30-kDa proteins are closely related but that the third 30-kDa protein is not. The ***p30*** gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified E. ***canis*** . Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 of E. ***canis*** . To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ***ehrlichiosis*** , the immunoreactions between rP30 and the whole purified E. ***canis*** antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly. . . in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ***ehrlichiosis*** . Cloning of multigenes encoding the 30-kDa major

outer membrane proteins of E. ***canis*** will greatly facilitate understanding pathogenesis and immunologic study of canine ***ehrlichosis*** and provide a useful tool for phylogenetic analysis.

IT Major Concepts
Bacteriology; Biochemistry and Molecular Biophysics; Infection

IT Diseases
ehrlichiosis : bacterial disease

IT Chemicals & Biochemicals
major outer membrane protein: 30-kDa, immunodominant; DNA

ORGN . . .
Mammalia, Vertebrata, Chordata, Animalia; Rickettsiaceae: Rickettsiales, Rickettsias and Chlamydias, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
DH82 (Canidae): dog macrophage cells; ***Ehrlichia*** - ***canis*** (Rickettsiaceae): pathogen

ORGN Organism Superterms
Animals; Bacteria; Carnivores; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Vertebrates